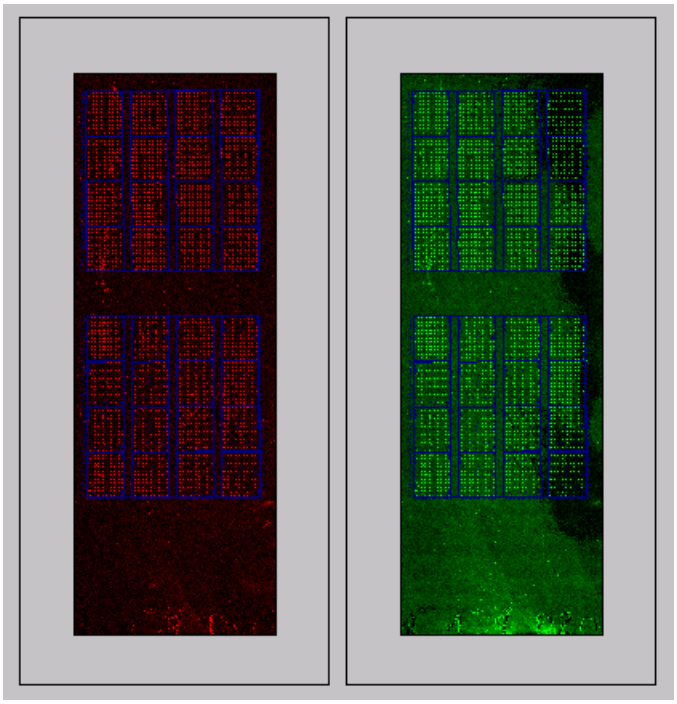
raytest

# *AIDA Microarray for Windows*

*User's Manual*



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# Introduction 1

## Overview

Array technology enormously speeds up the generation of new data from molecular biology experiments. It is of growing importance for many fields in life science and is on the way to enter applications in medical diagnosis. An essential tool to benefit from microarray experiments is a powerfull software evaluation tool.

AIDA Microarray is designed for the fast and reliable evaluation of quantitative and qualitative data from all kinds of array experiments in life science. It provides the optimized solution for slide evaluation with two or more fluorescence labeled targets. Two to six channels can be evaluated simultaneously, sharing the same highly efficient spot alignment algorithm and the automatic adaptation of the spot diameter.

With four simple steps precise data from array experiments can be generated:

1. Load Image
2. Load Template
3. Alignment & Calculation
4. Save and Export

Beside this ease of operation, AIDA Microarray has an unmatched flexibility in the adaptation to different array designs. Spotting patterns can be defined with multiple spotting probes and control probes. A great variety of background subtraction and normalization methods are available.

The Rating of Spot Value function allows you to check the quality of the array results manually as well as automatically. The results can be displayed using various data view options, including

- Schematic Histogram view,
- Schematic Display view,
- Scattered Plot view.

In addition, results can be filtered, automatically saved, exported to databases or transferred for further processing to the AIDA Array Clusterer  software tool.

# 1

# Starting Microarray

**To select the Microarray evaluation mode:**

1 Click the **Evaluation** button in the toolbar:

*Figure 1-1 Evaluation Button on the Main Toolbar*

A dialog appears offering you a list of available evaluation modes. The list entries reflect the installed AIDA application modules.

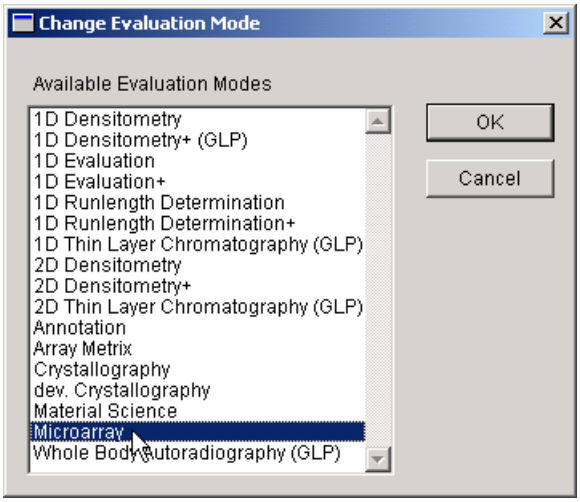



*Figure 1-2 Change Evaluation Mode Dialog*

2 Select the **Microarray** list item and click **OK**.

---

**Note –** The **Microarray** evaluation mode is only available if you purchased the AIDA Microarray module.

---

# Using Microarray 2

## Data Windows in Microarray

In addition to the AIDA standard data windows **Image** and **Histogram** the following data windows are available in the **Microarray** evaluation mode:

- Array Result Table
- Probe Table
- Array Scatter Plot
- Probe Scatter Plot
- Duplicate Correlation
- Schematic Display
- Schematic Histogram
- Repetition Deviation
- Analysis Table

To access these data windows, choose the appropriate entry from the **View** menu.

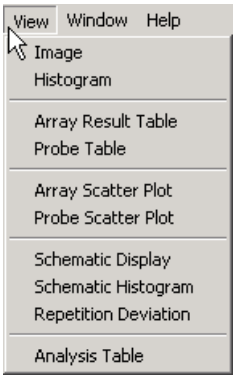



*Figure 2-1 View Menu*

# Image Window in Microarray

All data are evaluated from the **Image** window. It displays the different channels of a multi-channel image in a single view.

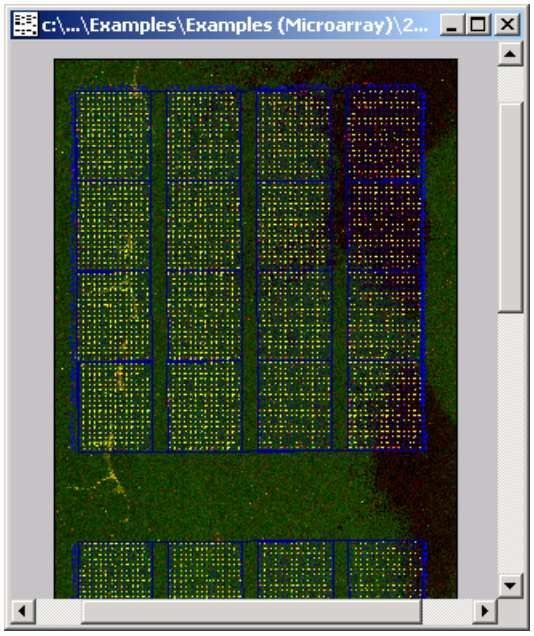



*Figure 2-2 Image Window Example*

Using the **Channel Display** controls of the **Display Control** toolbox, you can specify how the channels are displayed in the **Image** windows: Select the **Overlaid in false colors**, **Tile horizontal** or **Tile vertical** button to show all channels superimposed on each other in false color display, display each channel in a different window pane side by side or on top of each other respectively.

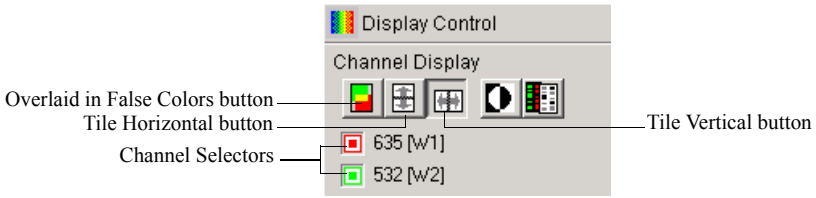



*Figure 2-3 Channel Display Buttons of the Display Control Toolbox*

You can show/hide channels using the channel selectors on the **Channel Display** panel.

Although the **Image** window might not always be on top, it is always open. The title bar of the **Image** window shows the full file path of the image.

To copy the content of the **Image** window to the clipboard, select the **Copy Content** option from the **Edit** menu.

## Shortcut Menu of the Image Window

**Cut**, **Copy**, **Paste** and **Delete** are used to manipulate overlays (for example array overlays, text and arrows on the **Image** window).

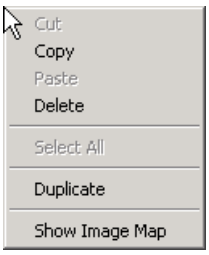



*Figure 2-4 Shortcut Menu (Image Window)*

Just click the Selection tool on the main toolbar, select the object you want to manipulate, and then choose the appropriate command from the shortcut menu.

*Figure 2-5 Selection Tool on the Main Toolbar*

**Select All** selects all overlays present in the active image.

The **Duplicate** option duplicates the array overlay.

**Show/Hide Image Map** shows/hides a navigator palette, which let you see the entire image as well as the portion you're zoomed into (indicated by a red viewing rectangle). You can stay zoomed in and move easily to another section by dragging the red rectangle within the paletteto another area.

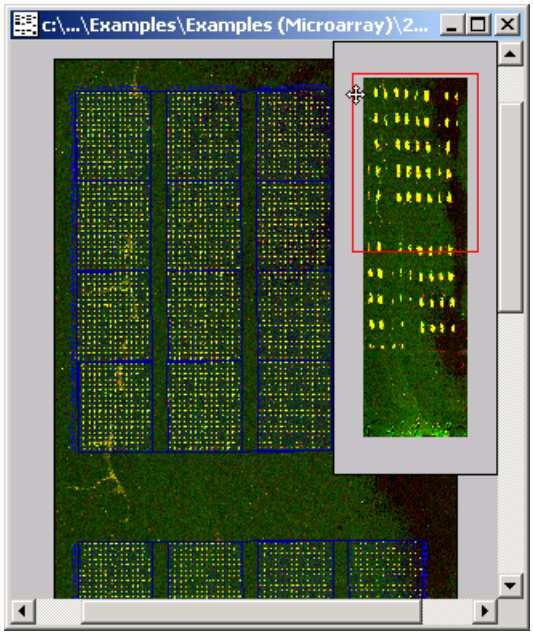



*Figure 2-6 Image Map Palette*

You can adjust the anchor position of the palette in the **Image** window by choosing the appropriate option from the shortcut menu of the palette.

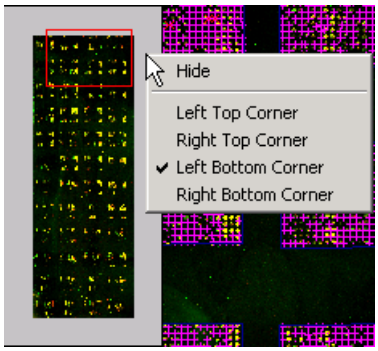



*Figure 2-7 Shortcut Menu of the Image Map Palette*

## Formatting Overlays

You can specify the font and array level you want to apply to the label overlays using the **Image Overlays** option of the **Options** menu.

After selecting **Image Overlays** from the **Options** menu, the **Image Overlays** dialog appears allowing you to select the level of array (**Label Array by**), font, font size, font style (regular, bold) for labeling.

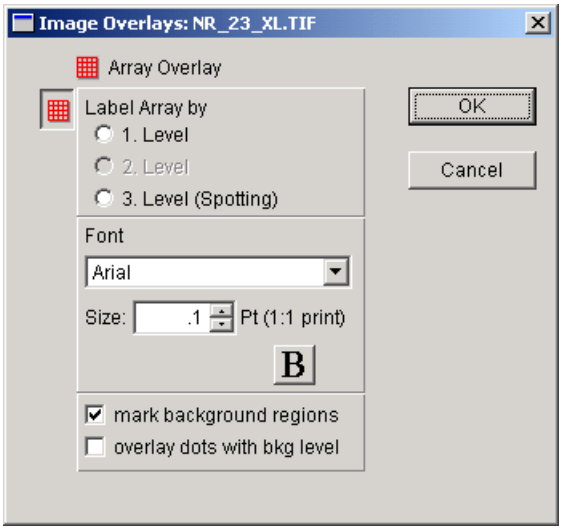
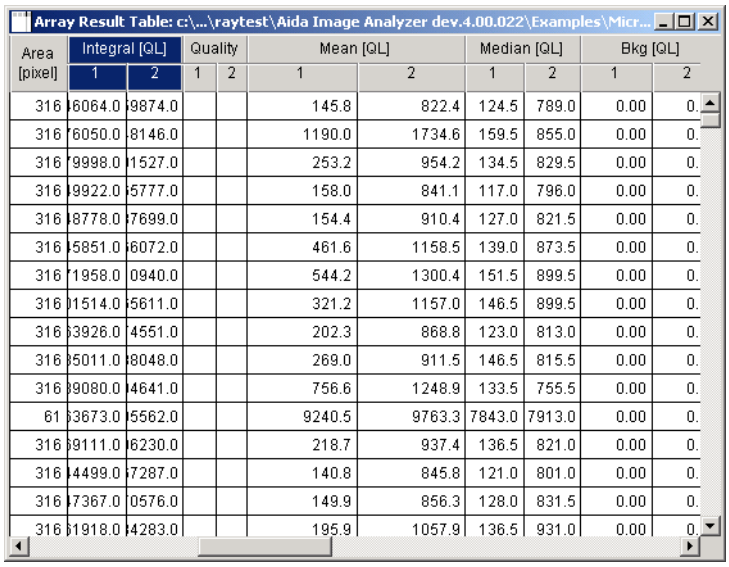



*Figure 2-8 Image Overlays Dialog*

In addition, you can show or hide the regions used for background calculation by selecting the **mark background regions** check box. Checking this option also sets marks for the local dot and grid ring as well as for mode of none spot, if the respective modes are selected. Further, the options shows or hides the background image regions if the **Global Image Regions** or **Weighted Image Regions** background method is chosen (for details about background definition, see the Background chapter below). If you check the **overlay dots with bkg level** option, each dot is overlaid by its background gray level.

# Array Result Table

This window shows the results of the array analysis in tabular form. It summarizes the measurements of all channels in a single data view. Values of multiple channels are listed in numbered child columns of a parent column.




| Area [pixel] | Integral [QL] 1 | Integral [QL] 2 | Quality 1 | Quality 2 | Mean [QL] 1 | Mean [QL] 2 | Median [QL] 1 | Median [QL] 2 | Bkg [QL] 1 | Bkg [QL] 2 |
|---|---|---|---|---|---|---|---|---|---|---|
| 316 | 6064.0 | 9874.0 | | | 145.8 | 822.4 | 124.5 | 789.0 | 0.00 | 0. |
| 316 | 6050.0 | 8146.0 | | | 1190.0 | 1734.6 | 159.5 | 855.0 | 0.00 | 0. |
| 316 | 9998.0 | 1527.0 | | | 253.2 | 954.2 | 134.5 | 829.5 | 0.00 | 0. |
| 316 | 9922.0 | 5777.0 | | | 158.0 | 841.1 | 117.0 | 796.0 | 0.00 | 0. |
| 316 | 8778.0 | 7699.0 | | | 154.4 | 910.4 | 127.0 | 821.5 | 0.00 | 0. |
| 316 | 5851.0 | 6072.0 | | | 461.6 | 1158.5 | 139.0 | 873.5 | 0.00 | 0. |
| 316 | 1958.0 | 0940.0 | | | 544.2 | 1300.4 | 151.5 | 899.5 | 0.00 | 0. |
| 316 | 1514.0 | 5611.0 | | | 321.2 | 1157.0 | 146.5 | 899.5 | 0.00 | 0. |
| 316 | 3926.0 | 4551.0 | | | 202.3 | 868.8 | 123.0 | 813.0 | 0.00 | 0. |
| 316 | 5011.0 | 8048.0 | | | 269.0 | 911.5 | 146.5 | 815.5 | 0.00 | 0. |
| 316 | 9080.0 | 4641.0 | | | 756.6 | 1248.9 | 133.5 | 755.5 | 0.00 | 0. |
| 61 | 3673.0 | 5562.0 | | | 9240.5 | 9763.3 | 7843.0 | 7913.0 | 0.00 | 0. |
| 316 | 9111.0 | 6230.0 | | | 218.7 | 937.4 | 136.5 | 821.0 | 0.00 | 0. |
| 316 | 4499.0 | 7287.0 | | | 140.8 | 845.8 | 121.0 | 801.0 | 0.00 | 0. |
| 316 | 7367.0 | 0576.0 | | | 149.9 | 856.3 | 128.0 | 831.5 | 0.00 | 0. |
| 316 | 1918.0 | 4283.0 | | | 195.9 | 1057.9 | 136.5 | 931.0 | 0.00 | 0. |

*Figure 2-9 Array Result Table Example*

**To display the Array Result Table:**

- Choose **View > Array Result Table**.

## Data Description

The possible columns of the **Array Result Table** are the following:

*Table 2-1 Array Result Table Columns*

| Columns | Short Description |
|---|---|
| **Spot Infomation** | |
| Index | Running table row number |
| Field | Field label (potential reference column for name table import) |

*Signal unit* and *length unit* = units of measurement, depending on the type of the imaging device used to obtain the image and the options set specified in the **Image Attributes** dialog.

*Table 2-1 Array Result Table Columns*

| Columns | Short Description |
|---|---|
| Block | Block label (potential reference column for name table import) |
| Spot | Spot label (potential reference column for name table import) |
| Row Index | Global grid position of a dot (row component; potential reference column for name table import) |
| Column Index | Global grid position of a dot (column component; potential reference column for name table import) |
| Field Row | Row position of the field within its array (potential reference column for name table import) |
| Field Column | Column position of the field within its array (potential reference column for name table import) |
| Block Row | Row position of the block within its field (potential reference column for name table import) |
| Block Column | Column position of the block within its field (potential reference column for name table import) |
| Spot Row | Row position of the spot within its block (potential reference column for name table import) |
| Spot Column | Column position of the spot within its block (potential reference column for name table import) |
| X [*pixel*] | X position of the spot measured of the left edge of the image |
| Y [*pixel*] | Y position of the spot measured of the upper edge of the image |
| Diameter [*pixel*] | Diameter of the spot |
| Area [*pixel*] | Number of pixels of the spot |
| Type | Type of spot (empty, guide, reference, background) |
| Name | Name of the probe to which the spot belongs (column name can be adjusted and cells can be linked to external database by the user via column shortcut menu command) |
| ID | ID number of the probe to which the spot belongs (column name can be adjusted and cells can be linked to external database by the user via column shortcut menu command) |
| <unused_1> | User-definable columns for name table import purpose (column name can be adjusted and cells can be linked to external database by the user via column shortcut menu command) |

*Signal unit* and *length unit* = units of measurement, depending on the type of the imaging device used to obtain the image and the options set specified in the **Image Attributes** dialog.

*Table 2-1 Array Result Table Columns*

| Columns | Short Description |
|---|---|
| <unused_2> | User-definable columns for name table import purpose (column name can be adjusted and cells can be linked to external database by the user via column shortcut menu command) |
| **Spot measurement values listed for each channel** | |
| Integral [*signal unit*] | Sum of the pixel intensity values of the spot |
| Mean [*signal unit*] | Mean of the pixel intensity values of the spot |
| Median [*signal unit*] | Median of the pixel intensity values of the spot |
| Bkg [*signal unit*] | Background intensity per pixel of a spot (see the "Background Settings" section of this manual for details) |
| Integral SD | Standard deviation of the pixel intensity values of the spot |
| Bkg SD | Standard deviation of the background pixels of the spot (see the "Background Settings" section of this manual for details) |
| Integral-Bkg [*signal unit*] | Background-corrected integral value |
| Mean-Bkg [*signal unit*] | Background-corrected mean value |
| Median-Bkg [*signal unit*] | Background-corrected median value |
| Saturated [*pixel*] | Number of saturated pixels |
| Homogeneity [%] | Homogeneity value (see the "Rating Settings" section of this manual for details) |
| Probe Median [*signal unit*] | Median of the measurement values of all repetitions in a probe. The measurement category used to calculate the median is the category which is used for normalisation (Integral, Mean, Median (-Bkg)) |
| Difference [*signal unit*] | Measurement value of the spot minus Probe Median |
| Rel. Difference [%] | Difference divided by Probe Median |
| Quality | Quality flag |
| Normalized | Normalized measurement value of the spot (see the "Normalization Settings" section of this manual for details) |
| Log Normalized | Binary logarithm of the Normalized value |
| Bkg Normalized | Normalized background value of the spot |
| Reference [*signal unit*] | Reference value used for normalization (see the "Normalization Settings" section of this manual for details). |

*Signal unit* and *length unit* = units of measurement, depending on the type of the imaging device used to obtain the image and the options set specified in the **Image Attributes** dialog.

*Table 2-1 Array Result Table Columns*

| Columns | Short Description |
|---|---|
| **The following columns are only available if a comparison with a master channel was performed** | |
| Ratio | Ratio of the normalized values, with Ratio Cut Off values applied |
| Log Ratio | Binary logarithm of the Ratio value |
| Expression | Value of the Ratio column encoded according to the following scheme:<br>**On**: result value of master within background range *and* client's result value above background<br>**Off**: result value of master above background and client's result value within background range<br>***Ratio***: ratio value > 1<br>***-1/Ratio***: ratio value < 1 |

*Signal unit* and *length unit* = units of measurement, depending on the type of the imaging device used to obtain the image and the options set specified in the **Image Attributes** dialog.

You can select the number and type of columns you want to display using the shortcut menu of any column in the window (see below).

**To change the column order:**

1. Click the heading of the column to be moved, hold down the mouse button and drag to the desired position in the table.
2. Release the mouse button.

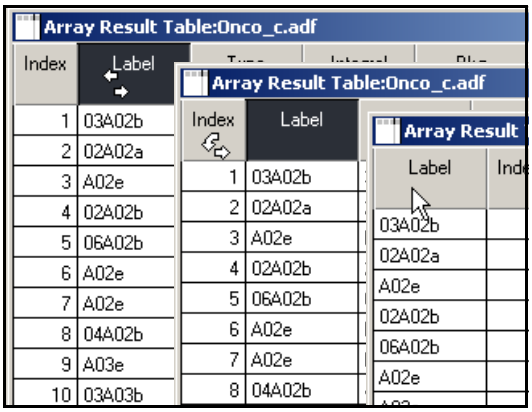



*Figure 2-10 Changing the Column Order*

**To change the column width:**

1. Point to the border of the column whose width you want to change.
2. Drag the border to the appropriate direction.
3. Release the mouse button.

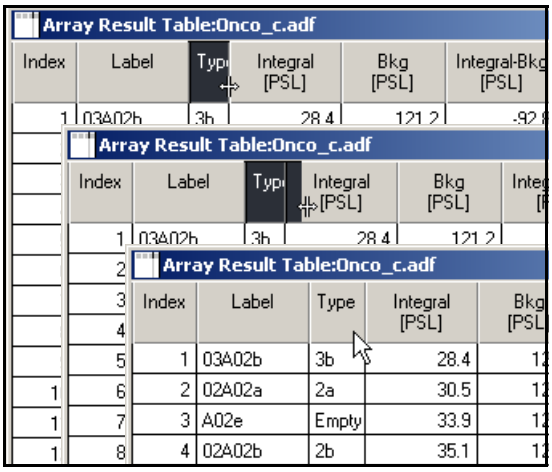



*Figure 2-11 Changing Column Width*

## Shortcut Menu of the Array Result Table

Each column in the **Array Result Table** has a shortcut menu attached.

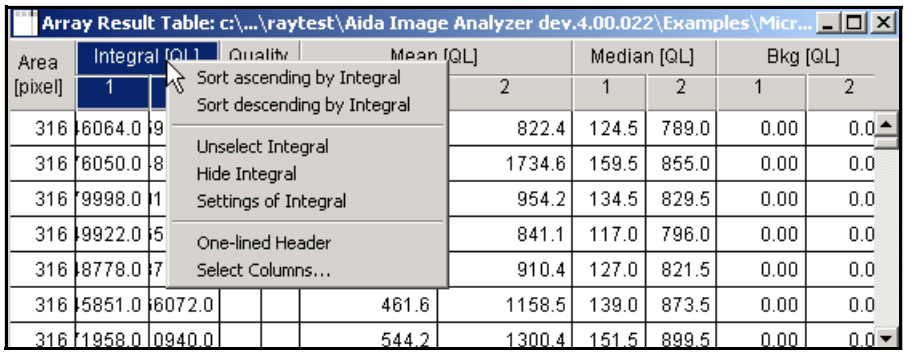



*Figure 2-12 Shortcut Menu (Array Result Table)*

The table can be sorted by any value column in ascending order (A to Z or zero to 9) or descending order (Z to A or 9 to zero). Just choose the appropriate menu item (**Sort ascending by**/**Sort descending by**)

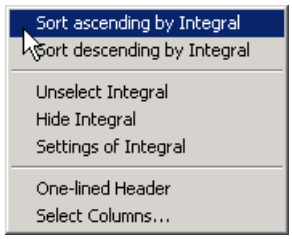



*Figure 2-13 Sorting Table*

This is especially useful for finding highest intensity values and for identifying the highest absolute or relative deviations in double dotted samples for quality control.

The **Select/Unselect** options of the shortcut menu are used to select/unselect a column to export. The column heading of selected columns are highlighted.

To hide a column, select the **Hide** option from its shortcut menu. You can recover hidden columns using the **Select Columns** option (see below).

To specify the decimal digits for the value output in a column, choose **Settings of** from the shortcut menu and enter the value for the decimal digits in the dialog that appears.

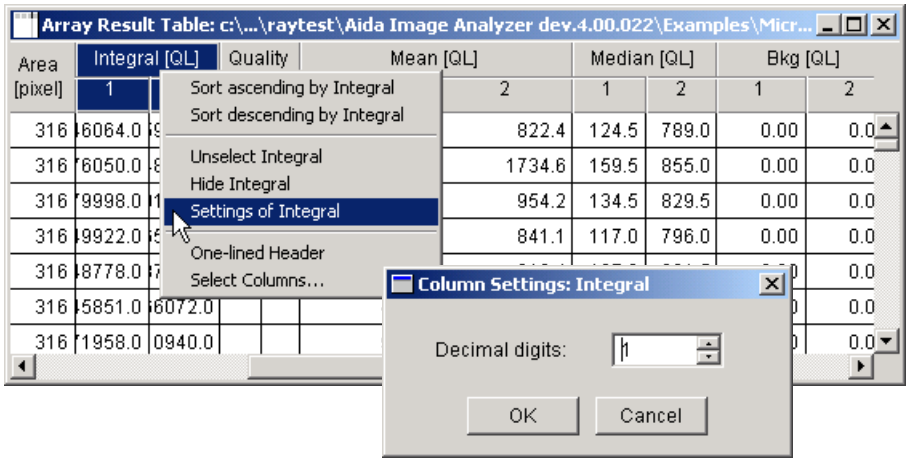



*Figure 2-14 Specifying Decimal Digits Display*

Note that the behavior of the **Settings of** option depends on the column you right-click: Columns whose cell contents is usually provided via name table import, that is the **Name** and the **ID** column as well as the two user-definable columns with the default name **<unused>**, have **Column Settings** dialog boxes totally different from the ones provided by measurement value output columns.

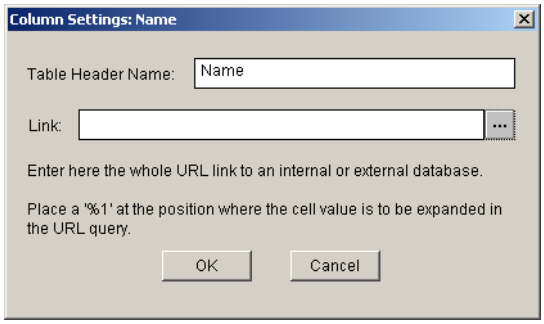

Column Settings: Name
Table Header Name:
Name
Link:
Enter here the whole URL link to an internal or external database.
Place a '%1' at the position where the cell value is to be expanded in the URL query.
OK
Cancel

*Figure 2-15 Column Settings Dialog (Name Column)*

Instead of decimal digit specifications, you can edit the column's default name and enter a URL (Uniform Resource Locator) to link the column's cells to internal or external databases (see the Importing Name Tables section for details)

Use the **One-lined Header/Two-lined Header** option to reduce or expand the column heading by one line (the second line indicates the units of measurement).

To select the columns that should be displayed in the table, choose the **Select Columns** option.

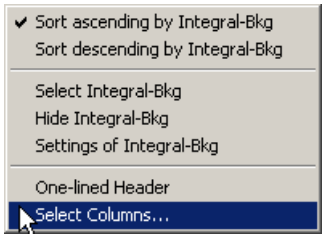



*Figure 2-16 Select Columns Command of the Shortcut Menu*

A dialog appears displaying two lists (titled: **Show Columns/Hide Columns**). Select the columns you want to show or hide and click the appropriate arrow symbol to transfer the items between the two lists. To unselect a particular item, just click it again.

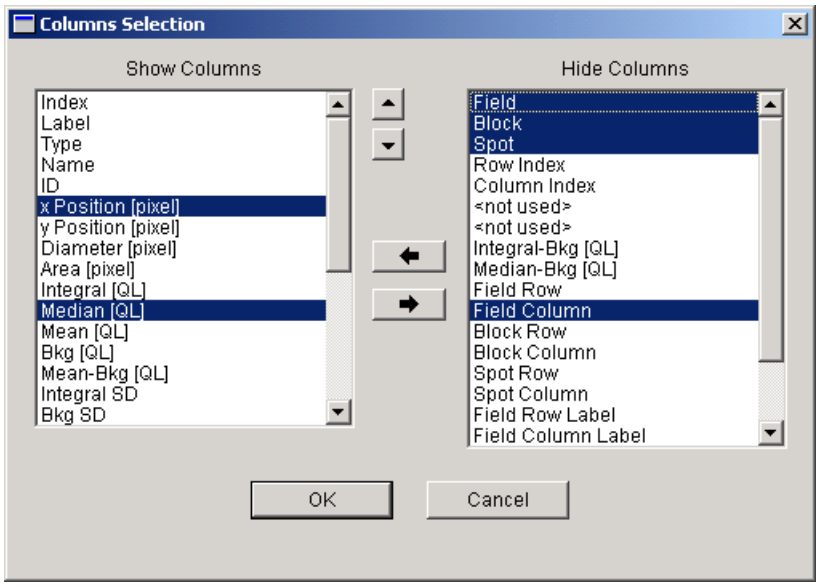
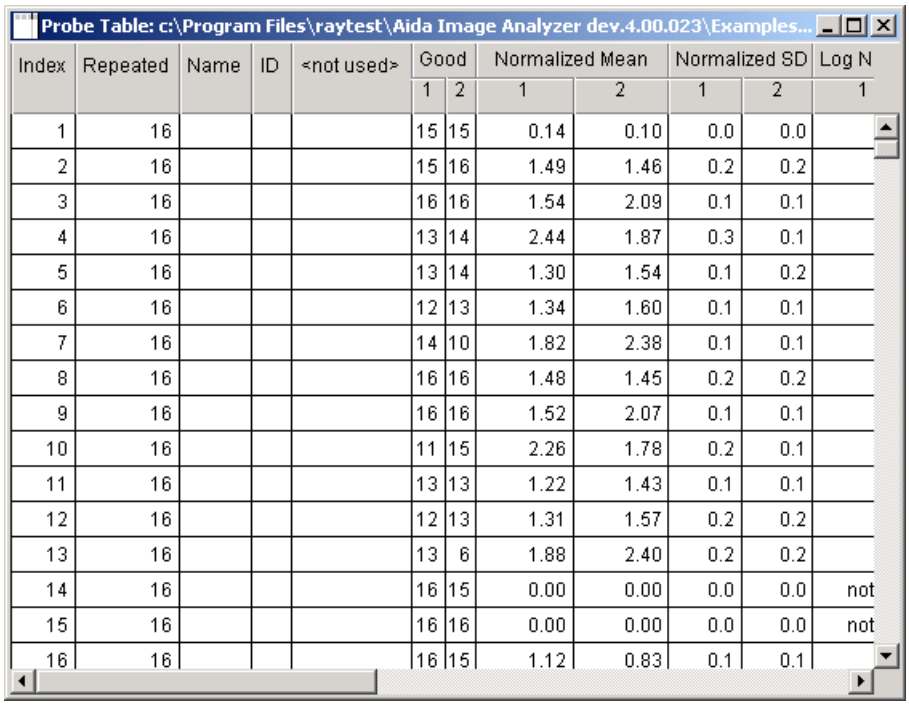



*Figure 2-17 Columns Selection Dialog*

# Probe Table

The **Probe Table** lists the averaged values of the repeated spots of a probe. Only “good” repetitions are used for the calculation of the averaged values. Values of multiple channels are listed in numbered child columns of a parent column.



Probe Table: c:\Program Files\raytest\Aida Image Analyzer dev.4.00.023\Examples...

| Index | Repeated | Name | ID | <not used> | Good | | Normalized Mean | | Normalized SD | | Log N |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | 1 | 2 | 1 | 2 | 1 | 2 | 1 |
| 1 | 16 | | | | 15 | 15 | 0.14 | 0.10 | 0.0 | 0.0 | |
| 2 | 16 | | | | 15 | 16 | 1.49 | 1.46 | 0.2 | 0.2 | |
| 3 | 16 | | | | 16 | 16 | 1.54 | 2.09 | 0.1 | 0.1 | |
| 4 | 16 | | | | 13 | 14 | 2.44 | 1.87 | 0.3 | 0.1 | |
| 5 | 16 | | | | 13 | 14 | 1.30 | 1.54 | 0.1 | 0.2 | |
| 6 | 16 | | | | 12 | 13 | 1.34 | 1.60 | 0.1 | 0.1 | |
| 7 | 16 | | | | 14 | 10 | 1.82 | 2.38 | 0.1 | 0.1 | |
| 8 | 16 | | | | 16 | 16 | 1.48 | 1.45 | 0.2 | 0.2 | |
| 9 | 16 | | | | 16 | 16 | 1.52 | 2.07 | 0.1 | 0.1 | |
| 10 | 16 | | | | 11 | 15 | 2.26 | 1.78 | 0.2 | 0.1 | |
| 11 | 16 | | | | 13 | 13 | 1.22 | 1.43 | 0.1 | 0.1 | |
| 12 | 16 | | | | 12 | 13 | 1.31 | 1.57 | 0.2 | 0.2 | |
| 13 | 16 | | | | 13 | 6 | 1.88 | 2.40 | 0.2 | 0.2 | |
| 14 | 16 | | | | 16 | 15 | 0.00 | 0.00 | 0.0 | 0.0 | not |
| 15 | 16 | | | | 16 | 16 | 0.00 | 0.00 | 0.0 | 0.0 | not |
| 16 | 16 | | | | 16 | 15 | 1.12 | 0.83 | 0.1 | 0.1 | |

*Figure 2-18 Probe Table Example*

**To display the Probe Table:**

1 Choose **View > Probe Table**.

## Data Description

The possible columns of the **Probe Table** are the following:

*Table 2-2 Probe Table Columns*

| Column | Short Description |
|---|---|
| Index | Running table row number |
| Name | Name of the probe, user-definable, corresponds to the **Name** column of the **Array Result Table** |
| ID | ID of the probe, user-definable, corresponds to the **ID** column of the **Array Result Table** |
| <not used_1> | User-definable columns, corresponds to the respective columns of the **Array Result Table** |
| <not used_2> | User-definable columns, corresponds to the respective columns of the **Array Result Table** |
| Repeated | Number of repetitions of the probe in the array, as determined by the spotting pattern |
| **Values listed for each channel; mean and standard deviation values are calculated using the "good" spot values of a channel from the result table of a probe.** | |
| Good | Number of repetitions *not* flagged for "Bad quality" |
| Normalized Mean | Mean of the Normalized values |
| Normalized SD | Standard deviation of the Normalized values |
| Log Normalized Mean | Mean of the Log Normalized values |
| Log Normalized SD | Standard deviation of the Log Normalized values |
| Ratio of Normalized Mean | Ratio of the Normalized Mean values with Ratio Comparison Settings applied |
| Expression | Ratio of Normalized Mean encoded according to the following scheme:<br>**On**: value of master within background range *and* client's value above background<br>**Off**: value of master above background and client's value within background range<br>***Ratio***: ratio value > 1<br>***-1/Ratio***: ratio value < 1 |

*Signal unit* and *length unit* = units of measurement, depending on the type of the imaging device used to obtain the image and the options set specified in the **Image Attributes** dialog.

*Table 2-2 Probe Table Columns*

| Column | Short Description |
|---|---|
| Log Ratio Mean | Mean of the Log Ratio values |
| Log Ratio SD | Standard deviation of the Log Ratio values |
| Delta Log Normalized | Log Normalized value of the channel minus Log Normalized value of the master channel |

*Signal unit* and *length unit* = units of measurement, depending on the type of the imaging device used to obtain the image and the options set specified in the **Image Attributes** dialog.

# Array Scatter Plot

In the **Array Scatter Plot** the Normalized or Log Normalized spot values of the measurement channels are plotted against the spot values of the master channel. The values of **Min. Expression** (red and green lines), **Max Ratio** (yellow lines), and **Bkg Cut Off** (yellow colored Bkg dots) configurable via the **Ratio Comparison** panel of the **Array Definition** toolbox are represented.

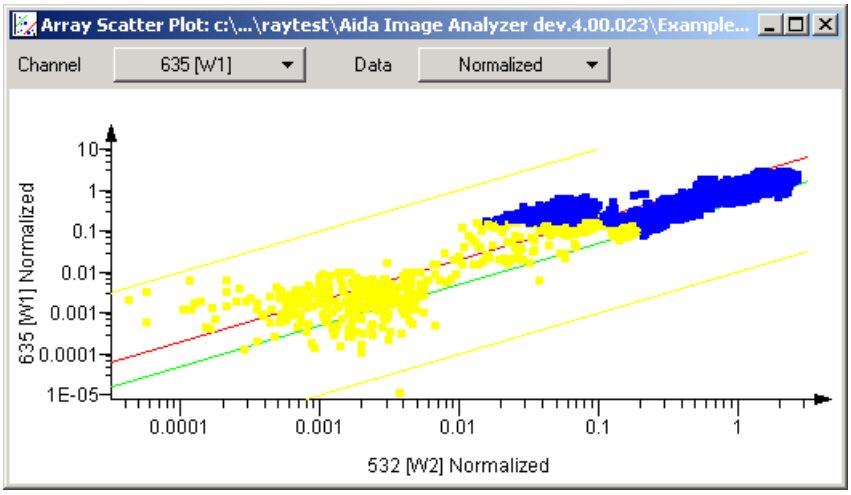



*Figure 2-19 Array Scatter Plot Example*

**To open the Array Scatter Plot window:**

- Choose **View > Array Scatter Plot**.

The measurement channel you want to plot against the master channel can be selected using the **Channel** menu of the **Array Scatter Plot** window. The data type can be selected via the **Data** menu of the **Array Scatter Plot** window.

For a more detailed view of the plot, the **Array Scatter Plot** window provides a zoom function:

**To zoom the scatter plot:**

1 Click the Selection tool on the main toolbar.

*Figure 2-20 Selection Tool on Main Toolbar*

**2** Drag one of the axis.

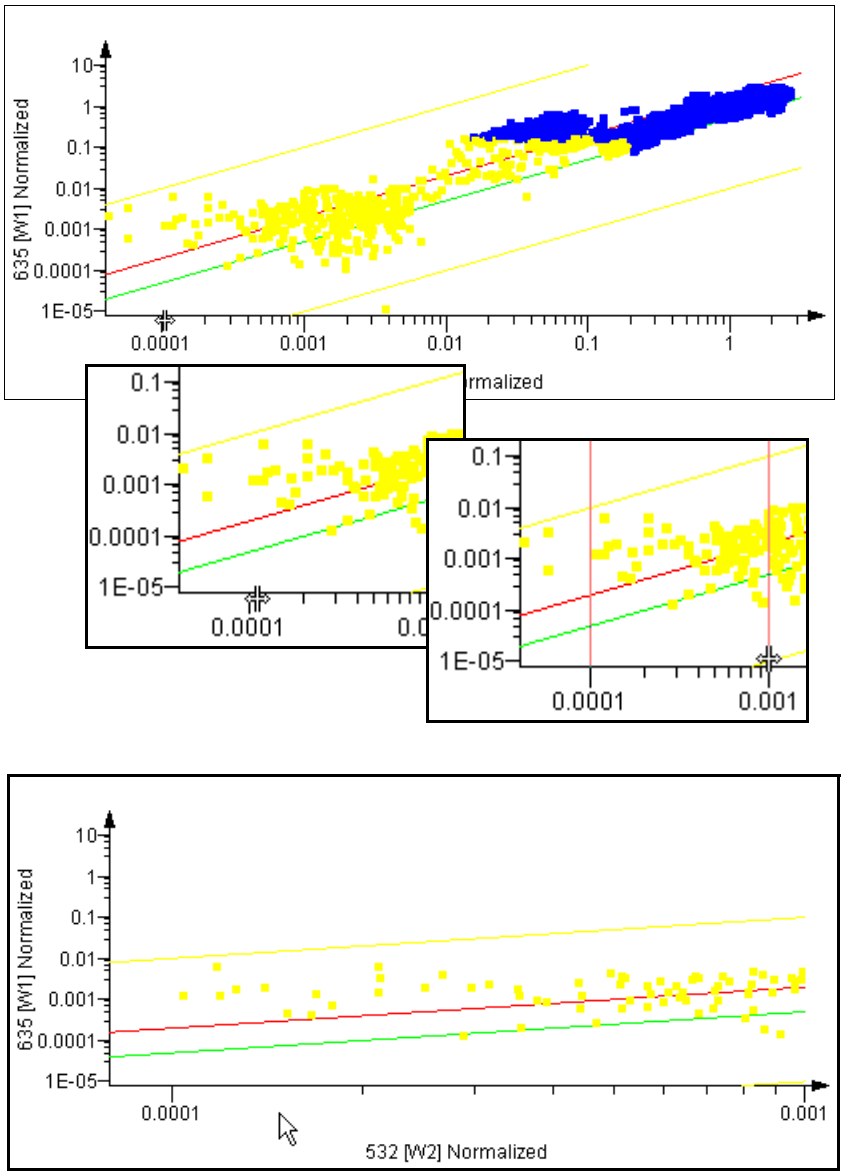



*Figure 2-21 Zooming Array Scatter Plot*

**Alternative procedure:**

1 Click the Zoom tool on the main toolbar.

*Figure 2-22 Zoom Tool on the Main Toolbar*

2 Draw a rectangle around the area of the plot you want to see in more detail.

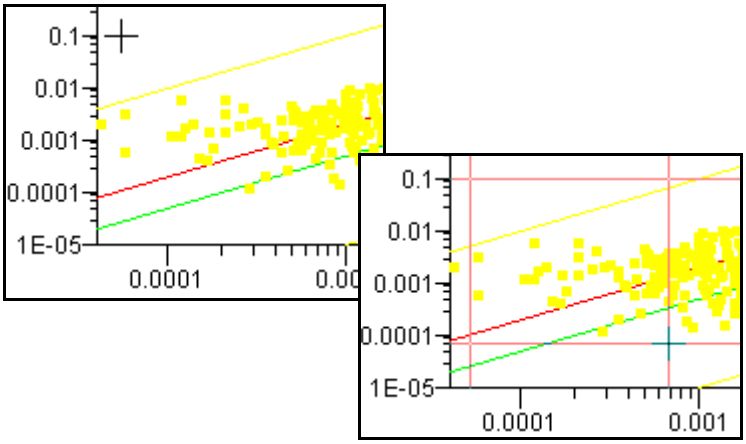



*Figure 2-23 Zooming Array Scatter Plot (Alternative Procedure)*

To review the data values of dots shown in the **Array Scatter Plot** window, click the dot. The coordinates of the dot are highlighted, together with all repetitions of the dot.

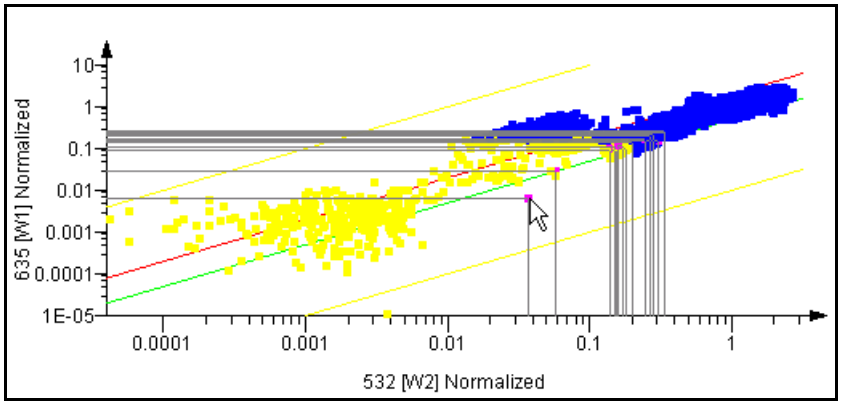



*Figure 2-24 Reviewing Array Scatter Plot Values*

## Shortcut Menu of the Array Scatter Plot

The shortcut menu of the **Array Scatter Plot** window provides you with two options: **Scales** and **Grid**.

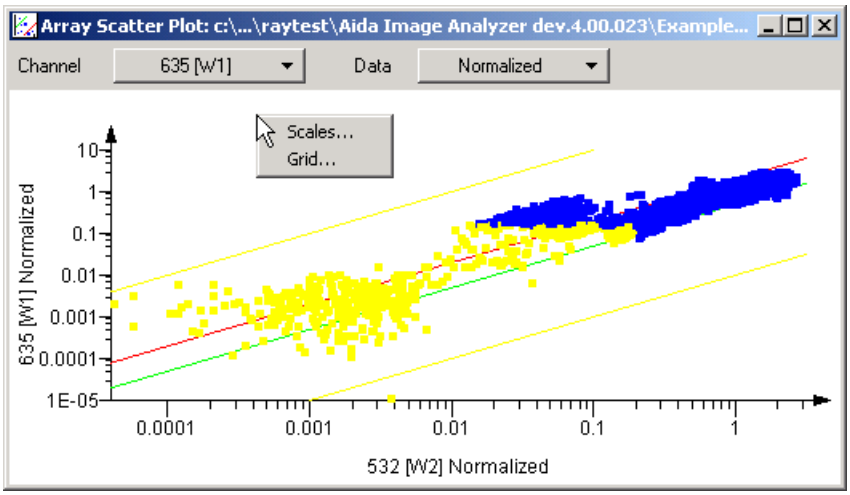



*Figure 2-25 Array Scatter Plot Window (Shortcut Menu)*

The **Scales** option allows you to configure the graphical output. After selecting the **Scales** option, the **Date Scaling** dialog opens.

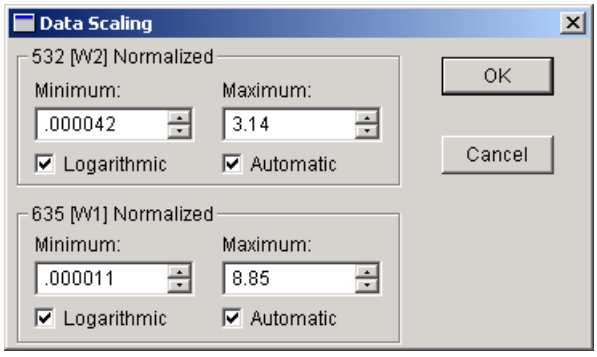



*Figure 2-26 Data Scaling Dialog*

Here the **Minimum** and **Maximum** value of the x-axis (**532 [W2] Normalized**) and y-axis (**635 [W1] Normalized**) can be selected. In addition, you can enable logarithmic representation and automatic scaling of either axis separately by checking the respective option (**Logarithmic** and **Automatic**) below the **Minimum** and **Maximum** spin boxes.

Choosing the **Grid** option opens the **Grid** dialog box, which allows you to lay grids over both axis that are an extension of either the big or the small ticks on the axes.

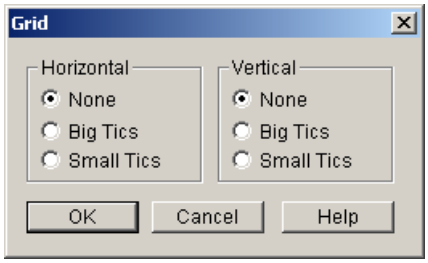



*Figure 2-27 Grid Dialog Box*

## Probe Scatter Plot

In the **Probe Scatter Plot** window the probe data (the averaged values of the spots of a probe)of the measurement channels are plotted against the spot data of the master channel. The values of **Min. Expression** (red and green lines), **Max Ratio** (yellow lines), and **Bkg Cut Off** (yellow colored Bkg dots) configurable via the **Ratio Comparison** panel of the **Array Definition** toolbox are represented.

The mean of the Log ratios of the data sets (gray line) and the data/mean thresholds (color-coded lines) configurable via the **Array Schematic** toolbox are represented.

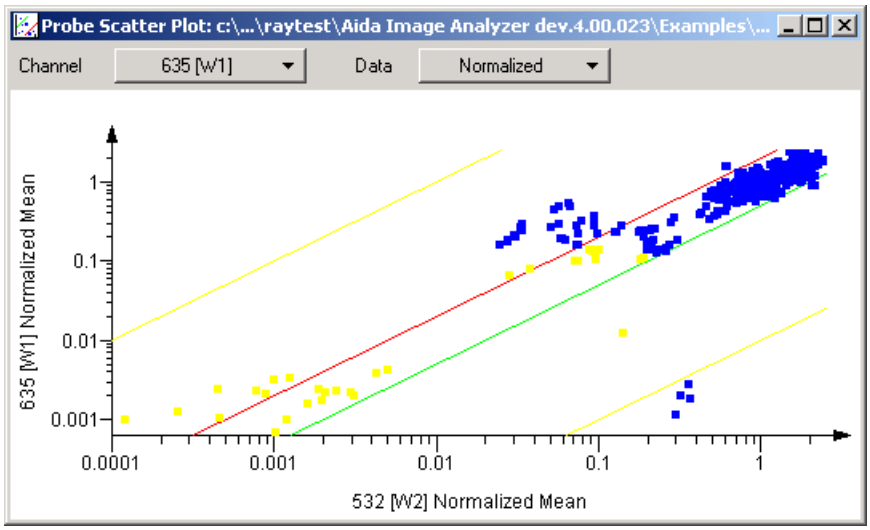



*Figure 2-28 Probe Scatter Plot Example*

**To open the Probe Scatter Plot:**

- Choose **View** > **Probe Scatter Plot**.

The **Probe Scatter Plot** window provides the same set of display controls as the **Array Scatter Plot**window, so please refer to the respective description in the Array Scatter Plot section for further details.

## Schematic Display Window

The **Schematic Display** window provides you with a schematic drawing of the array. In this data view, the mesurement values of the spots of a channel are color-coded. Any value of any channel can be selected using the **Schematic Display** panel of the **Array Schematic** toolbox. All data within a specific data range are represented by spots of identical color.

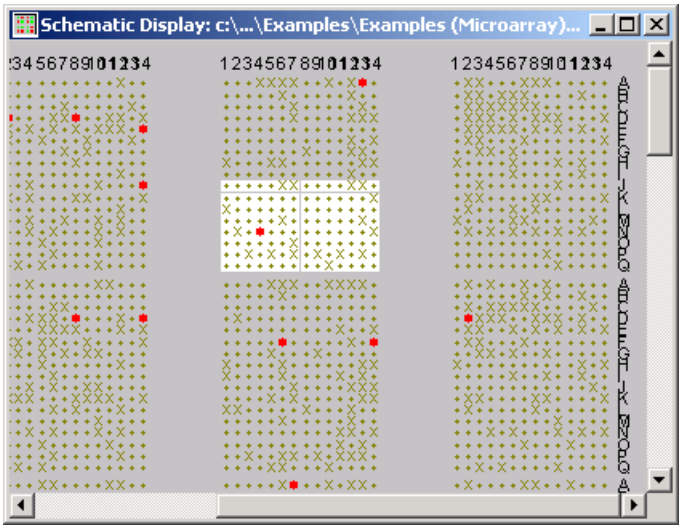



*Figure 2-29 Schematic Display Window Example*

Alternatively, the deviation of the spots to the mean value of data of interest can be coded by color. Values excluded from the Schematic Display via the **Exclude from schematic** option of the **Array Schematic** toolbox (empty dots, guide dots or bad dots) appear in gray and are marked with a cross to avoid confusion with “real” gray scale values.

**To open the Schematic Display window:**

- Choose **View > Schematic Display**.

Because of the permanent linkage between all data windows, you can use the **Schematic Display** window to easily navigate through the array on the **Image** window in order to select spots or spot groups.

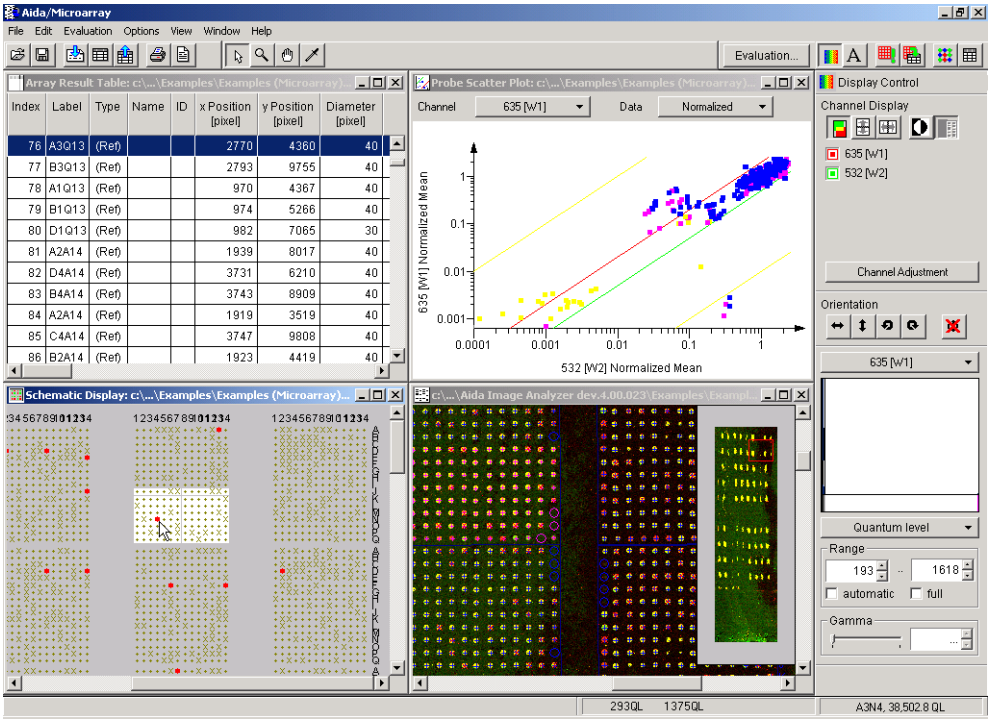

Aida/Microarray
File Edit Evaluation Options View Window Help
Evaluation...
Array Result Table: c:\...\Examples\Examples (Microarray)...
Index Label Type Name ID x Position [pixel] y Position [pixel] Diameter [pixel]
76 A3Q13 (Ref) 2770 4360 40
77 B3Q13 (Ref) 2793 9755 40
78 A1Q13 (Ref) 970 4367 40
79 B1Q13 (Ref) 974 5266 40
80 D1Q13 (Ref) 982 7065 30
81 A2A14 (Ref) 1939 8017 40
82 D4A14 (Ref) 3731 6210 40
83 B4A14 (Ref) 3743 8909 40
84 A2A14 (Ref) 1919 3519 40
85 C4A14 (Ref) 3747 9808 40
86 B2A14 (Ref) 1923 4419 40
Probe Scatter Plot: c:\...\Examples\Examples (Microarray)...
Channel 635 [W1] Data Normalized
635 [W1] Normalized Mean
532 [W2] Normalized Mean
Schematic Display: c:\...\Examples\Examples (Microarray)...
Aida Image Analyzer dev-4.00.023\Examples\Exampl...
Display Control
Channel Display
635 [W1]
532 [W2]
Channel Adjustment
Orientation
635 [W1]
Quantum level
Range
193 1618
automatic full
Gamma
293QL 1375QL
A3N4, 38,502.8 QL

*Figure 2-30 Interlinked Windows*

# Schematic Histogram Window

This data window shows a graphical representation of the distribution of values for a given data type (selected via the controls of the **Schematic Display** panel on the **Array Schematic** toolbox) as displayed in the **Schematic Display** window.

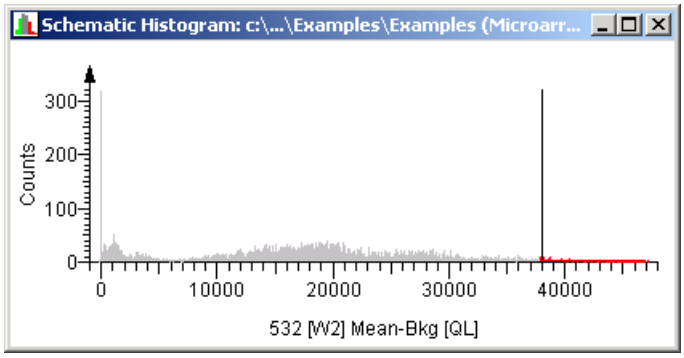



*Figure 2-31 Schematic Histogram Window Example*

**To open the Schematic Display window:**

- Choose **View > Schematic Display**.

Using the **Schematic Display** panel on the **Array Schematic** toolbox, you can configure the **Schematic Histogram** (and the **Schematic Display**) window to display certain ranges of data values in different colors.

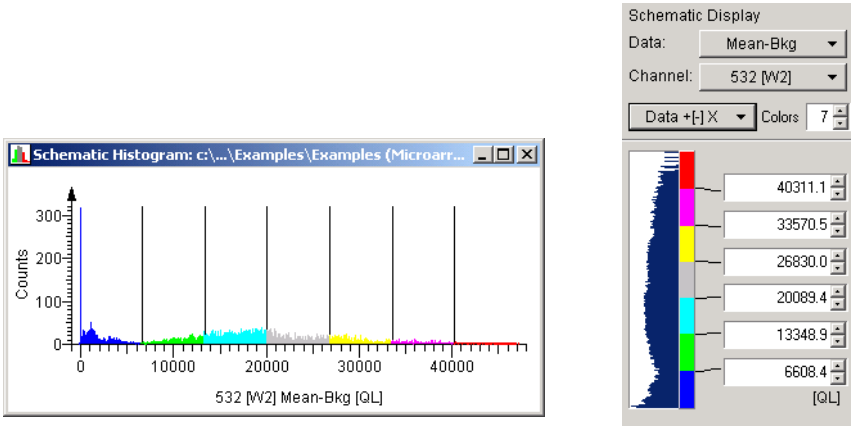



*Figure 2-32 Defining Color Coding Scheme*

For a more detailed view of the histogram, the **Schematic Histogram** window provides a zoom function similar to the one provided by the **Array Scatter Plot** window (see the Array Scatter Plot section for a detailed description of how to use the zoom function).

## Shortcut Menu of the Schematic Histogram Window

The shortcut menu of the **Schematic Histogram** window provides one option: **Scales**.

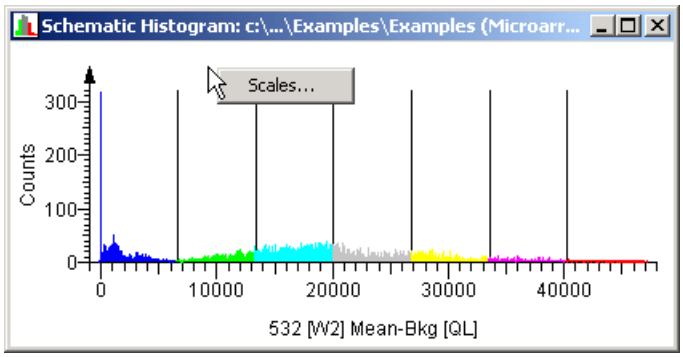



*Figure 2-33 Schematic Histogram Window (Shortcut Menu)*

The **Scales** option allows you to configure the graphical output. After selecting the **Scales** option, the **Date Scaling** dialog opens.

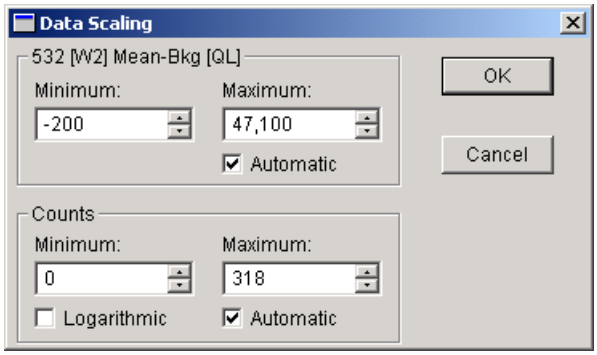



*Figure 2-34 Data Scaling Dialog*

Here the **Minimum** and **Maximum** value of the x-axis (**532 [W2] Mean-Bkg [QL]**) and y-axis (**Counts**) can be selected. In addition, you can enable automatic scaling of either axis separately and logarithmic representation for the y-axis only by checking the respective option (**Logarithmic** and **Automatic**) below the **Minimum** and **Maximum** spin boxes.

# Repetition Deviation Window

This data window shows a scatter diagram of the measurement values of a channel for all repetitions defined on the array.

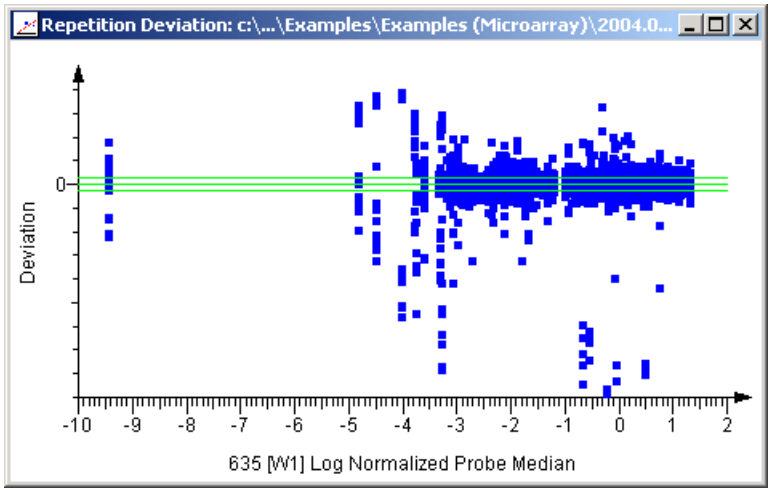



*Figure 2-35 Repetition Deviation Window*

**To open the Repetiton Deviation window:**

- Choose **View > Repetition Deviation**.

The type of the measurement values and the channel for which the scatter diagram is generated can be selected using the **Schematic Display** panel of the **Array Schematic** toolbox.

**To specify the type of the measurement values to be used for the scatter diagram:**

- On the **Array Schematic** toolbox, select the desired data type from the **Data** menu of the **Schematic Display** panel.

  If you select **Integral**, **Background**, **Mean**, **Median**, **Integral-Bkg**, **Mean-Bkg**, **Median-Bkg**, or **Normalized**, for each spot, the x-axis indicates the median (of the repetitions in the probe) for the selected measurement value, whereas the y-axis indicates the relative difference between the spot's measurement value and the median value of the probe.

If you select **Log Normized**, or **Log Ratio**, for each spot, the x-axis indicates the median (of the repetitions in the probe) for the selected measurement value, whereas the y-axis indicates the absolute difference between the spot's measurement value and the median value of the probe.

If you select **Ratio**, for each spot, the x-axis indicates the ratio of the median values (of the repetitions in the probe) for the normalized values of both channels, whereas the y-axis indicates the relative difference between the spot's Ratio value and the ratio of the median Normalized values of the probe:

$$ration = \frac{n_1}{n_2}$$

where
$n_1$ is the Normalized value of the measurement channel, and
$n_2$ is the Normalized value of the master channel.

$$m_{ratio} = m_{n_1} / m_{n_2}$$

where

$m_{n_1}$ is the median (of the repetitions in the probe) for the Normalized values of the mesurement channel, and

$m_{n_2}$ is the median (of the repetitions in the probe) for the Normalized values of the master channel.

On the x-axis, the $m_{ratio}$ is indicated, on the y-axis, $(ratio - m_{ratio})/m_{ratio}$ is indicated.

**To specify the channel for which you want to display the repetition deviation:**

- On the **Array Schematic** toolbox, select the desired data type from the **Channel** menu of the **Schematic Display** panel.

# Analysis Table

In contrast to the **Array Result Table**, which lists the analysis results for the entire array, the **Analysis Table** is used to display analysis results for a subset of the array.

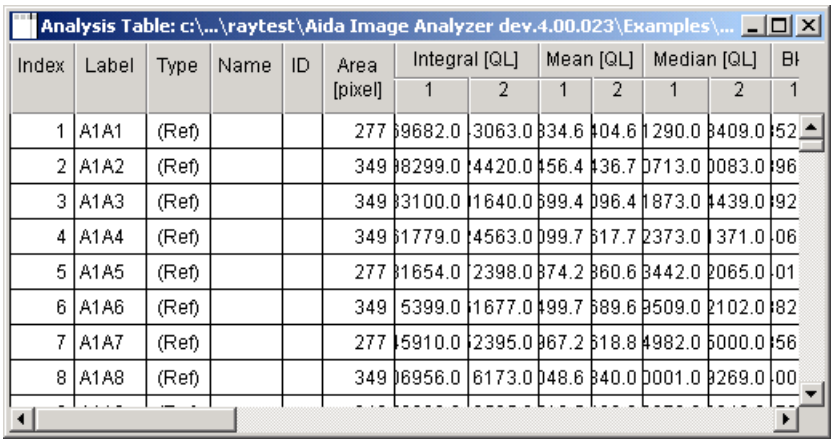



*Figure 2-36 Analysis Table Window Example*

You can define those subsets in two different ways: manually or by filtering using user-defined rules (for details, see the Array Analysis Table Toolbox section of this manual).

**To open the Analysis Table:**

- Choose **View > Analysis Table**.

The **Analysis Table** window provides the same set of display controls as the **Array Result Table** window, so please refer to the respective description in the Array Result Table section for further details.

# Additional Toolboxes in Microarray

After selecting the **Microarray** evaluation mode, the standard AIDA toolbox set (**Display Control** and **Publishing Layout**) is expanded by four additional toolboxes:

- Array Definition
- Array Measurement
- Array Schematic
- Array Analysis Table

You can access these four additional toolboxes via the **Evaluation** menu or by clicking the appropriate button on the toolbar.

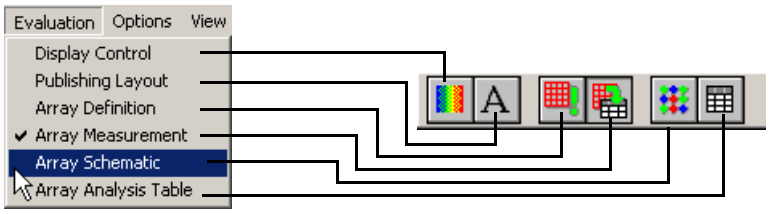



*Figure 2-37 Evaluation Menu-Toolbar Correspondences*

# Array Definition Toolbox

The **Array Definition** toolbox allows you to define high density array overlays.

To display the **Array Definition** toolbox:

On the main menu, click **Evaluation**, and then click **Array Definition**. Alternatively you can select the **Array Definition** button on the toolbar.

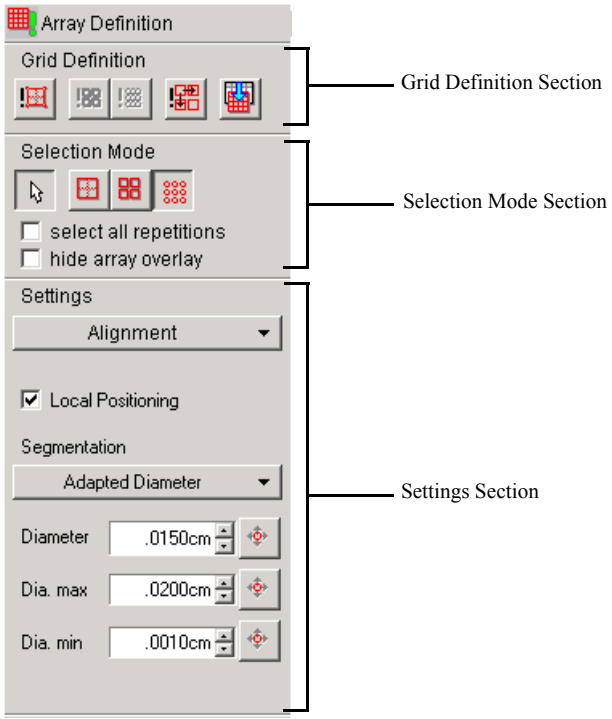



*Figure 2-38 Array Definition Toolbox*

## Grid Definition Section

In the **Grid Definition** section of the toolbox you can define new array levels or edit existing ones.

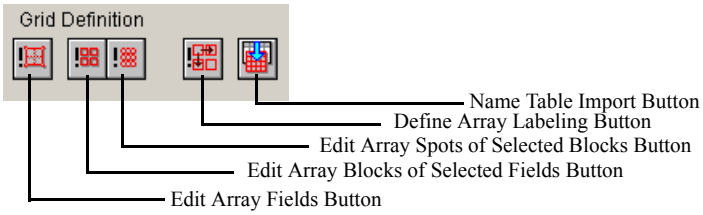

Grid Definition
Name Table Import Button
Define Array Labeling Button
Edit Array Spots of Selected Blocks Button
Edit Array Blocks of Selected Fields Button
Edit Array Fields Button

*Figure 2-39 Grid Definition Section Overview*

---

**Note –** In Array Easy, the functionality of the **Grid Definition** panel is restricted to editing the spot diameter of loaded predefined templates.

---

The **Edit Array Fields** button is used to define a frame for a new array overlay or to modify an existing one. Depending on whether you create a new array from scratch or edit an existing array overlay, the appearance of the toolbox changes.

The **Edit Array Blocks of Selected Fields** button is used to edit the second array level. The second array level is defined for selected fields of the first array level. If no fields are selected on the first array level, this option is disabled.

Use the **Edit Array Spots of Selected Blocks** button to edit the third array level. The third array level is defined for selected fields of the first or blocks of the second array level, depending on the previously defined array level structure. If no fields or blocks are selected, this option is disabled.

To label spots, click the **Define Array Labeling** button.

The **Name Table Import** option is intended for easy import of names and ID numbers of samples like clones, sequences, oligos and so on

A detailed description of the various options in this section of the toolbox is provided in the “Defining a New Template”section of this manual.

## Selection Mode Section

The **Selection Mode** section of the **Array Definition** toolbox provides a toolbar used to choose different array level selections.

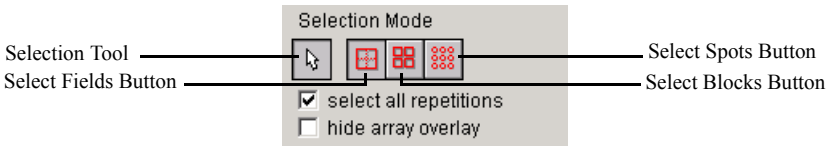



*Figure 2-40 Selection Mode Section Overview*

Clicking on the **Select Fields** button activates the selection of the fields on the first array level, **Select Blocks** activates the selection of the blocks, and **Select Spots Level** is used to select the spots in the array.

Check the **select all repetitions** option, if you want to enable the joint selection of a spot and its related duplicated spot.

To hide the array overlay, check the **hide array overlay** option.

## Settings Section

The **Settings** section provides you with a menu for choosing various array parameter settings:

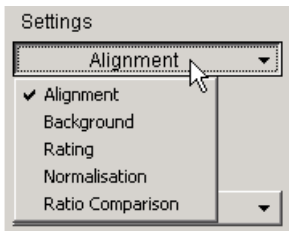



*Figure 2-41 Settings Menu*

For a detailed description of the parameter settings, see the “Setting Parameters” section.

# Array Measurement Toolbox

The **Array Measurement** toolbox provides you with all tools necessary to evaluate arrays.

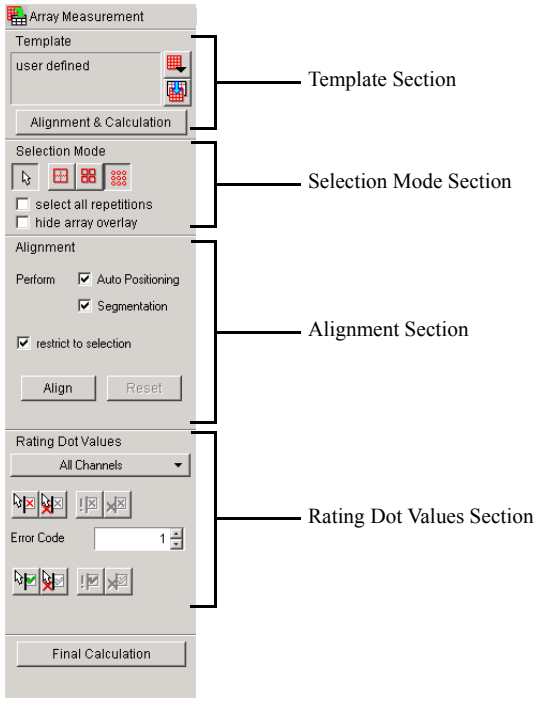



*Figure 2-42 Array Measurement Toolbox Overview*

**To display the Array Measurement toolbox:**

- Choose **Evaluation** > **Array Measurement**.

Alternatively you can select the **Array Measurement** button on the toolbar

## Template Section

The Template section of the Array Measurement toolbox is used to import predefined array templates.

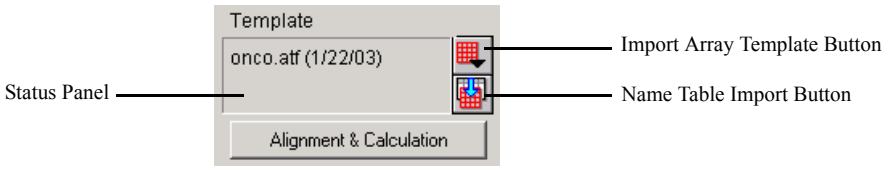



*Figure 2-43 Template Section Overview*

To import a predefined array template from the template library shipped with AIDA Microarray, click the **Import Array Template** button.

The **Name Table Import** option is intended for easy import of names and ID numbers of samples like clones, sequences, oligos and so on

The status panel shows the name of the currently applied (imported) template.

The **Alignment & Calculation** button is used to start the automated alignment and calculation (measurement, background calculation, rating, normalization) process. The Alignment and Calculation process is applied to the entire array regardless of the current selection and **restrict to selection** settings.

For a detailed description of the various options provided by this section of the toolbox, see the “Defining a New Template” section of this manual.

## Selection Mode Section

As with the corresponding section of the **Array Definition** toolbox, the **Selection Mode** section of the **Array Measurement** toolbox provides a toolbar used to choose different array level selections.

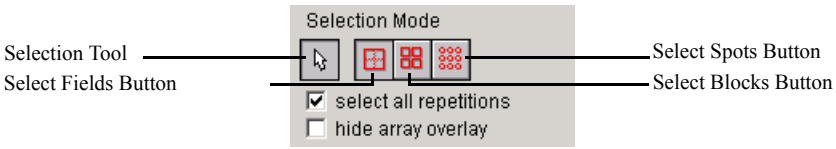



*Figure 2-44 Selection Mode Section Overview*

Clicking on the **Select Fields** button activates the selection of the fields on the first array level, **Select Blocks** activates the selection of the blocks in the fields, and **Select Spots** is used to select the spots (third level) in the blocks or fields.

Check the **select all repetitions** option, if you want to enable the joint selection of a spot and its related duplicated spot. To hide the array overlay, check the **hide array overlay** option.

## Alignment Section

With the controls in the **Alignment** section you can set parameters for automated alignment processes used to match the position of the defined array spots with the real position of the dots in the array. This alignment process is consequently accomplished by the integration of the intensity values of each spot..

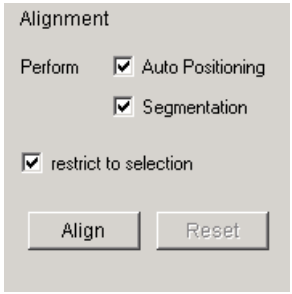



*Figure 2-45 Alignment Section*

The **Alignment** section provides you with the following controls:

- **Auto Positioning**: Check this option to enable the automatic positioning of the array.
- **Segmentation**: Check this option to enable automatic segmentation of the spots.
- If the **restrict to selection** option is enabled, the automated alignment process is applied to selected part of the array only.

  (See the “Standard Evaluation Procedure” chapter for further information
- The **Align** button is used to start the alignment process.
- The **Reset** button restores the selected part of the array to its initial state. To undo the alignment, choose the **Undo** command from the **Edit** menu.

## Rating Dot Values Section

The quality of spot results can be influenced by a large number of factors (for example spotting accuracy, existence of artefacts, overshining effects). The **Rating Dot Values** section of the **Array Measurement** toolbox allows you to identify spots with problematic integration values manually.

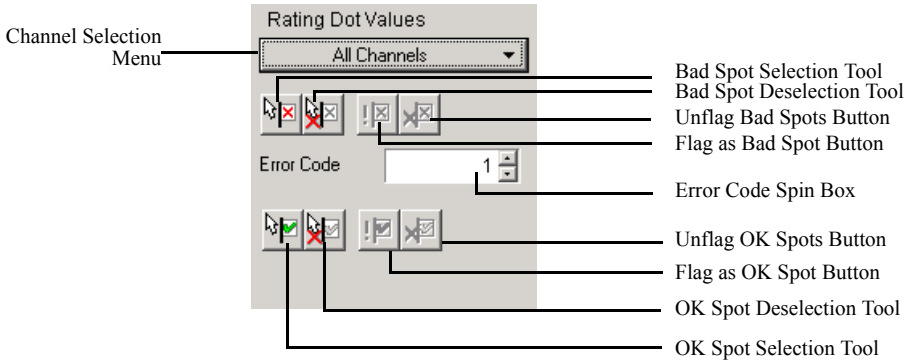



*Figure 2-46 Rating Dot Values Section*

The **Rating Dot Values** section contains the following controls:

- The channel selection menu is used to select the channel for dot rating.
- The **Bad Spot Selection** tool is used to flag the integration value of a spots as not reliable. Just select the **Bad Spot Selection** tool and click the spot you want to mark.
- To remove the mark from a spot, choose the **Bad Spot Deselection** tool and click the spot, you want the flag to be removed.
- Use the **Flag as Bad Spot** button to turn previously selected spots into bad spots.
- Clicking the **Unflag Bad Spots** button removes bad spot flagging from all currently selected spots.
- To relate an error code to the flagging of the spots, choose a code number (1 to 7) from the **Error Code** spin box. The error code is displayed for each bad spot in the **Quality** row of the **Array Result Table** and the **Analysis Table**.
- The **OK Spot Selection** tool is used to explicitly flag the integration value of a previously rated spots as reliable (**ok**). Just select the **OK Spot Selection** tool and click the spot you want to mark.
- To switch back to the original rating mark of a previously OK marked spot, choose the **OK Spot Deselection** tool and click the spot, you want the flag to be removed.

- Use the **Flag as OK Spot** button to explicitly turn previously selected spots into OK spots.
- Clicking the **Unflag OK Spots** button removes OK spot flagging from all currently selected spots.

### Final Calculation Button

Selecting the **Final Calculation** button, all remaining calculations required for integration, background determination, automatic rating, and normalization are performed.

(For further details, see the “Alignment Settings” chapter below).

## Array Schematic Toolbox

With the **Array Schematic** toolbox you can configure the settings for the schematic analysis of the array.

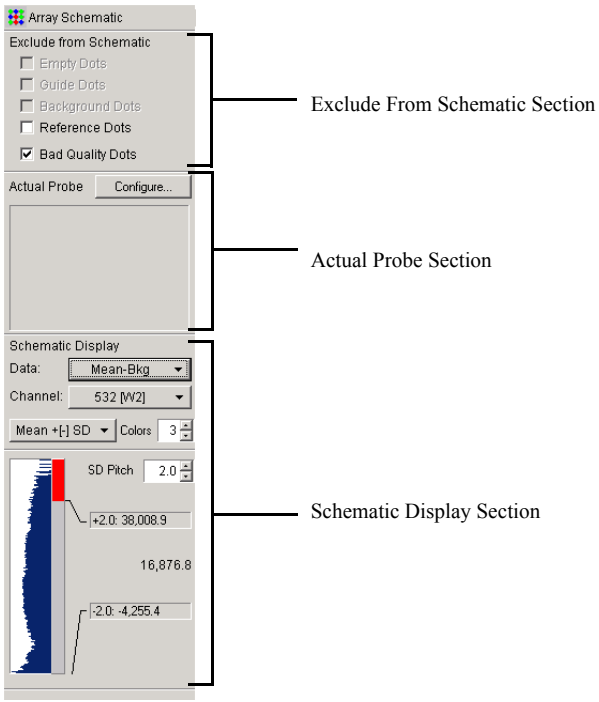



*Figure 2-47 Array Schematic Toolbox Overview*

## Exclude From Schematic Section

In the **Exclude from Schematic** section of the **Array Schematic** toolbar you can select some dot types for exclusion from the schematic analysis of the array. These types are: **Empty Dots**, **Guide Dots**, **Background Dots**, **Reference Dots** and **Bad Quality Dots**.

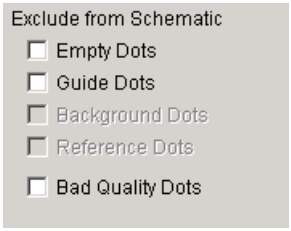



*Figure 2-48 Exclude From Schematic Section*

Values excluded via the **Exclude from Schematic** option appear in gray in the **Schematic Display** window and are marked with a cross to avoid confusion with "real" gray scale values.

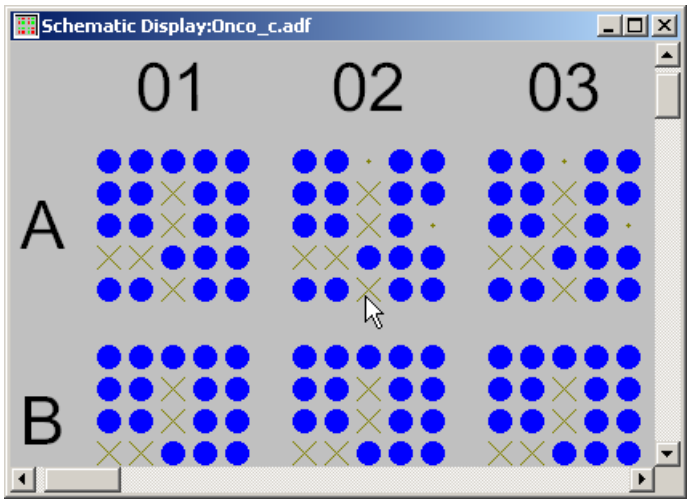



*Figure 2-49 Dots Excluded From Schematic*

Note that enabling the **Exclude from Schematic** option results in a complete exclusion of the specified dots from any calculation (mean and standard deviation calculation as well as Schematic Histogram).

## Actual Probe Section

In the message panel below the **Exclude from Schematic** section, the name of the selected probe as stated in the **Array Result Table** is shown together with its label and the value for the measured data (selected in the **Data** drop-down menu of the **Schematic Display** section).

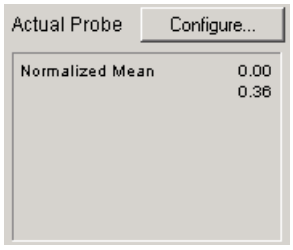



*Figure 2-50 Actual Probe Section*

You can select the type of data to be displayed on the **Actual Probe** panel by clicking the **Configure** button and selecting/deselecting the appropriate data types from the list in the **Column Selection** dialog.

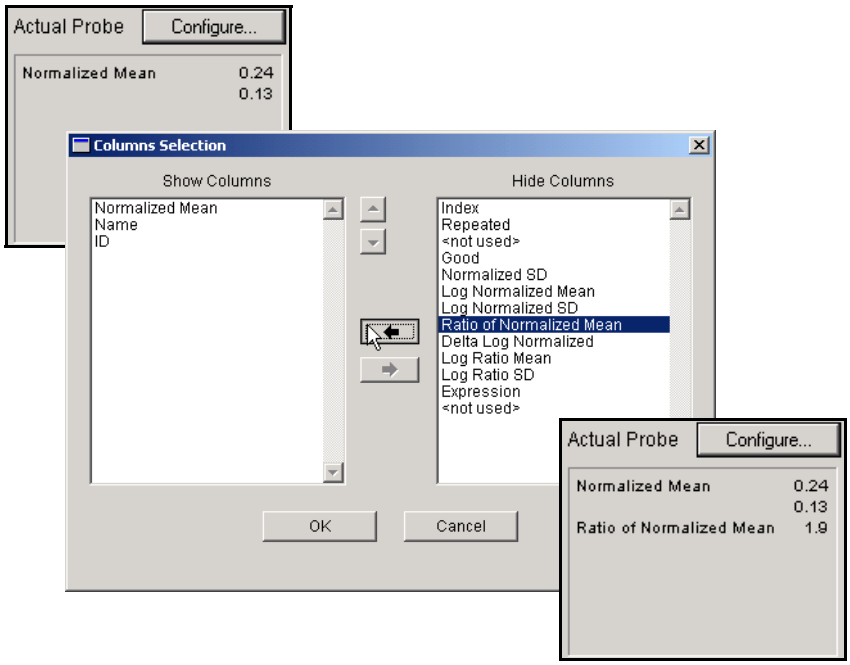



*Figure 2-51 Configuring Actual Probe Data Display*

## Schematic Display Section

The **Schematic Display** section is used to configure the display options for the **Schematic Display**, the **Schematic Histogram**, and the **Repetition Deviation** window.

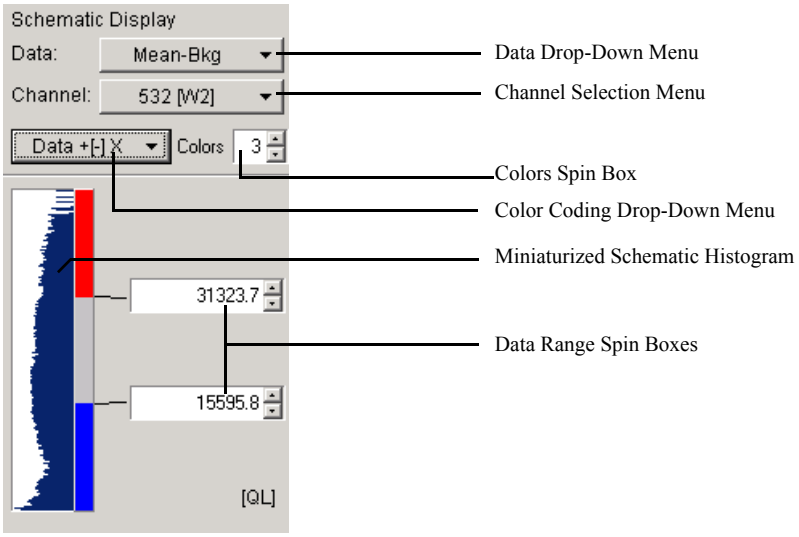



*Figure 2-52 Schematic Display Section Overview*

Select the measurement value and channel to be displayed in the **Schematic Display** window from the **Data** and **Channel** drop-down menu respectively.

To configure the color output of the **Schematic Display** window, choose the number of colors (3, 5, or 7) you want the data to be reduced to using the **Colors** spin box.

Specify the color coding scheme for the data via the drop-down menu displayed to the left of the **Colors** spin box. Either the data of interest within a specified range are displayed in one of the colors (**Data +[-] X** option) or the deviation to the mean value is coded by the colors (**Mean +[-] SD** option).

If the **Data +[-] X** option is selected, set the data range for each color by dragging the border of the respective color bar in the miniaturized **Schematic Histogram** with the mouse or by entering the explicit values. If the **Mean +[-] SD** option is selected, this

feature is disabled. Instead, the deviation of the spots to the mean value of the data of interest is given in units of 10th standard deviation (SD). Enter the step size in the **SD Pitch** spin box.

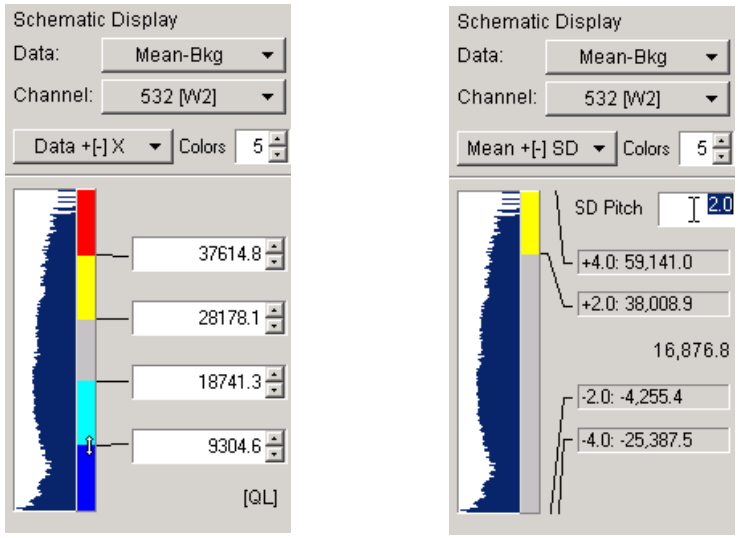



*Figure 2-53 Setting Data Range (left) and Specifying Step Size (right)*

# Array Analysis Table Toolbox

With the **Array Analysis Table** toolbox you can configure the settings for the **Analysis Table** window.

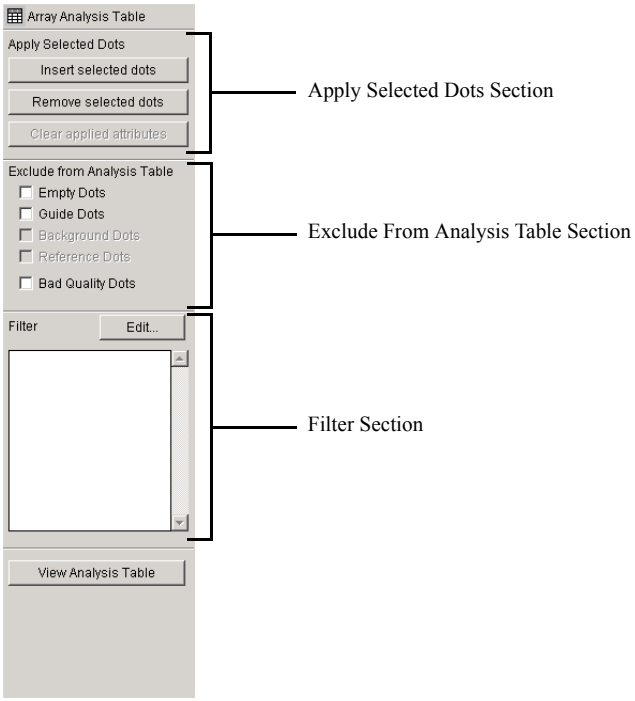



*Figure 2-54 Array Analysis Table Toolbox Overview*

## Apply Selected Dots Section

The controls of this panel are used to manually add values to or remove values from the **Analysis Table**.

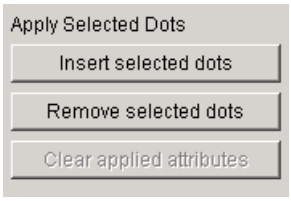



*Figure 2-55 Apply Selected Dots Section*

## Exclude From Analysis Table Section

In the **Exclude from Analysis Table** section of the **Array Analysis Table** toolbox you can select some dot types for exclusion from the **Analysis Table**. These types are: **Empty Dots**, **Guide Dots**, **Background Dots**, **Reference Dots** and **Bad Quality Dots**.

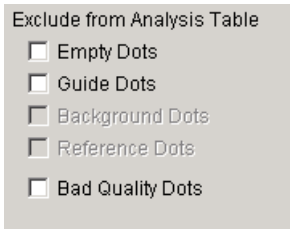



*Figure 2-56 Exclude From Analysis Table Section*

## Filter Section

This panel allows you to guide the automatic filtering process for analysis result values provided by AIDA Microarray.

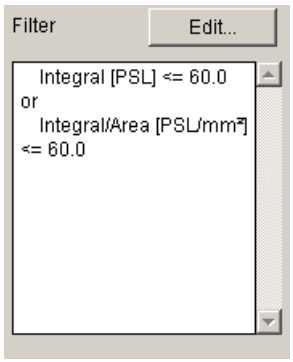



*Figure 2-57 Filter Section With Rules Assigned*

The automatic result value filtering process is based on rules defined by the user. There are no built-in default filtering rules, so unless you define a specific rule for filtering, no automatic filtering is applied. The filter to be specified consist of a list of conditions (**Alternatives**) concatenated by an OR operator. An Alternative is a AND-linked list of conditionals for evaluating the data values of a particular spot.

The filter rules you define are *Permission* rules: Values that *do not* meet the conditions stated in the rules are blocked from being displayed in the **Analysis Table** window.

**To enter a rule statement:**

1. Click the **Edit** button on the **Filter** panel.

   The **Array Analysis Table Clauses** dialog appears.

2. On the **Alternatives** panel, click **Add**.

   This adds the phrase **1. Clause is:** to the **Alternatives** list panel.

3. Then click the **Add** button on the Clauses panel.

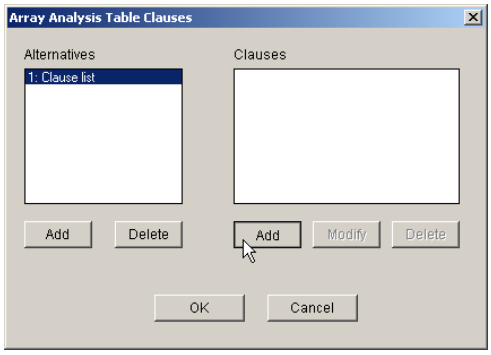



*Figure 2-58 Adding rule clauses*

This opens the **Analysis Table Clauses** dialog, which allows you to define your rule expression.

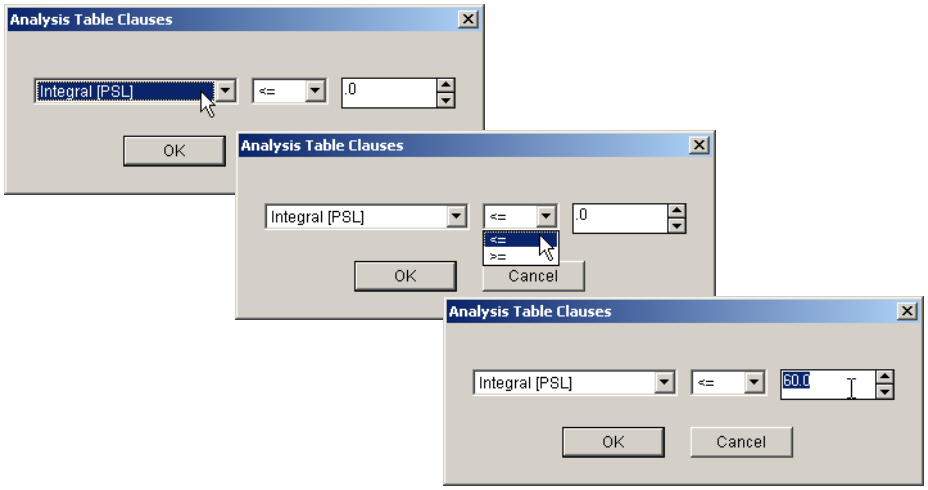



*Figure 2-59 Specifying Rule Expression*

# Publishing Layout Toolbox in Microarray

In Microarray the standard AIDA **Protocol Print** toolbar of the **Publishing Layout** toolbox is expanded by four additional buttons for specifying the print options for the **Probe Table**, **Array Scatter Plot**, **Probe Scatter Plot**, **Schematic Display**, **Schematic Histogram**, **Repetition Deviation**, and the **Array Analysis Table**:

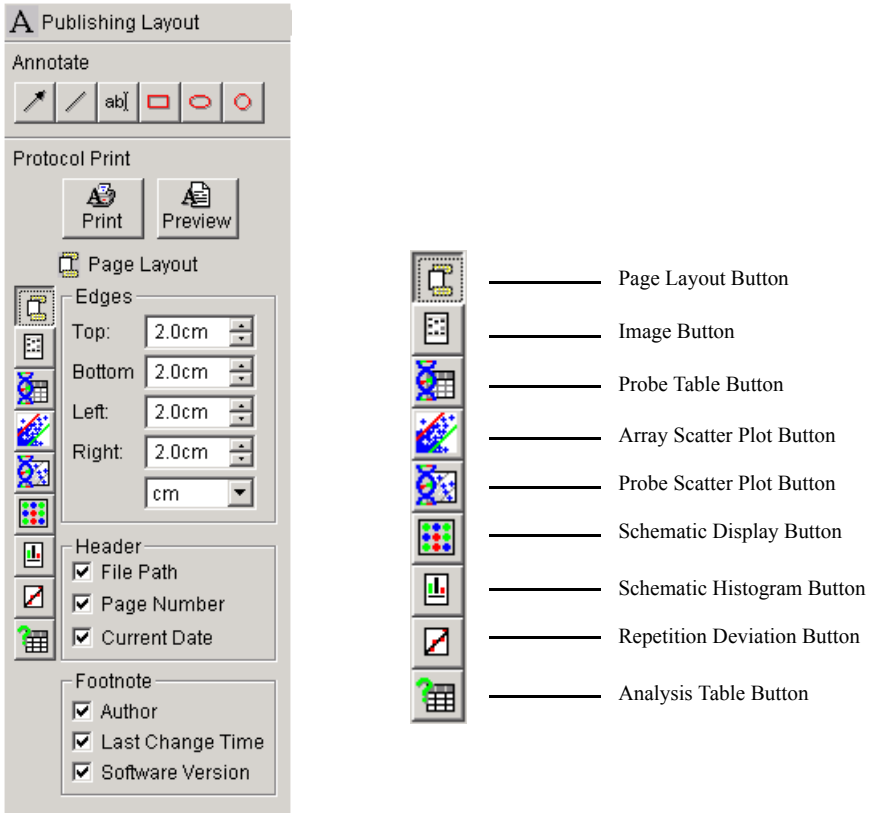



*Figure 2-60 Publishing Layout Toolbox and Protocol Print Toolbar*

## Print Options for Probe Table

Clicking the **Probe Table** button switches to the layout setting controls for the **Probe Table** window. To select the paper orientation (**Portrait** or **Landscape**), click the appropriate radio button in the **Format** section. In the **Print** section the scope for the print output can be specified. To enable printing of the table, check the **Table** box and specify the appropriate output extent using the **Full Table**/**Rows above** radio buttons.

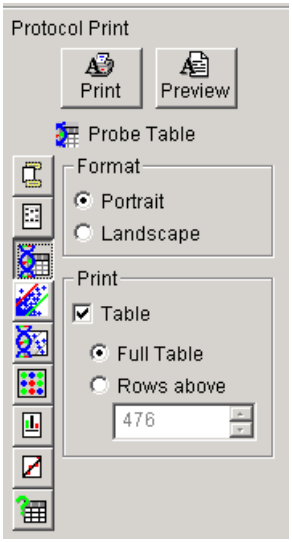



*Figure 2-61 Array Analysis Table Print Options*

# Print Options for Array Scatter Plot

The **Array Scatter Plot** button on the **Protocol Print** toolbar allows you to configure the layout setting for the **Array Scatter Plot** window. You can select the paper orientation (**Portrait** or **Landscape**) by clicking the appropriate radio button in the **Format** section.The **Graphic** option in the **Print** section enables or suppresses the print output of the graphic displayed in the **Array Scatter Plot** window.

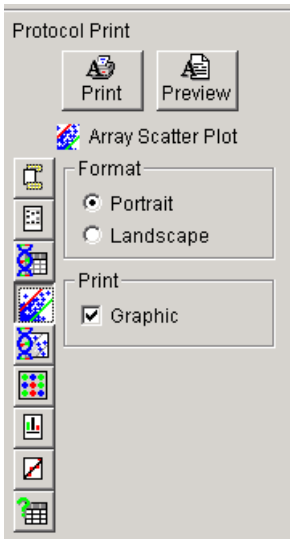



*Figure 2-62 Array Scatter Plot Print Options*

# Print Options for Probe Scatter Plot

The **Probe Scatter Plot** button on the **Protocol Print** toolbar allows you to configure the layout setting for the **Probe Scatter Plot** window. You can select the paper orientation (**Portrait** or **Landscape**) by clicking the appropriate radio button in the **Format** section.The **Graphic** option in the **Print** section enables or suppresses the print output of the graphic displayed in the **Probe Scatter Plot** window.

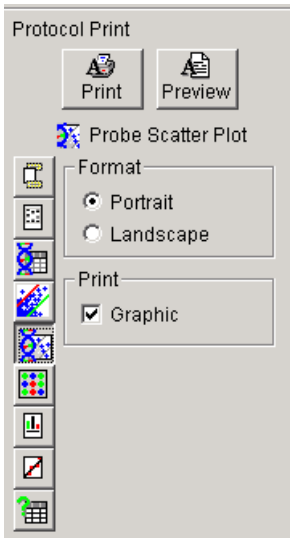



*Figure 2-63 Probe Scatter Plot Print Options*

# Print Options for Schematic Display

To configure the layout setting for the **Schematic Display** window, choose the **Schematic Display** button provided by the **Protocol Print** toolbar. As for **Probe Scatter Plot**, you can select the paper orientation (**Portrait** or **Landscape**) by clicking the appropriate radio button in the **Format** section.The only option in the **Print** section enables or suppresses the print output of the **Schematic Display** window.

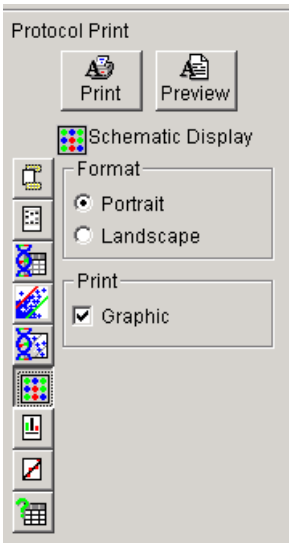



*Figure 2-64 Schematic Display Print Options*

# Print Options for Schematic Histogram

The **Schematic Histogram** button on the **Protocol Print** toolbar allows you to configure the layout setting for the **Schematic Histogram** window. You can select the paper orientation (**Portrait** or **Landscape**) by clicking the appropriate radio button in the **Format** section.The **Graphic** option in the **Print** section enables or disables the print output of the graphic displayed in the **Schematic Histogram** window.

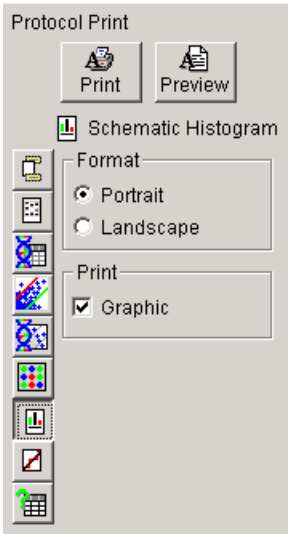



*Figure 2-65 Schematic Histogram Print Options*

## Print Options for Repetition Deviation

The **Repetition Deviation** button on the **Protocol Print** toolbar allows you to configure the layout setting for the **Repetition Deviation** window. You can select the paper orientation (**Portrait** or **Landscape**) by clicking the appropriate radio button in the **Format** section.The **Graphic** option in the **Print** section enables or disables the print output of the graphic displayed in the **Repetition Deviation** window.

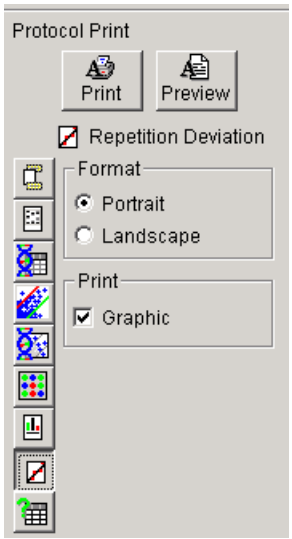



*Figure 2-66 Repetition Deviation Print Options*

## Print Options for Analysis Table

Clicking the **Analysis Table** button switches to the layout setting controls for the **Analysis Table** window. To select the paper orientation (**Portrait** or **Landscape**), click the appropriate radio button in the **Format** section. In the **Print** section the scope for the print output can be specified. To enable printing of the table, check the **Table** box and specify the appropriate output extent using the **Full Table**/**Rows above** radio buttons.

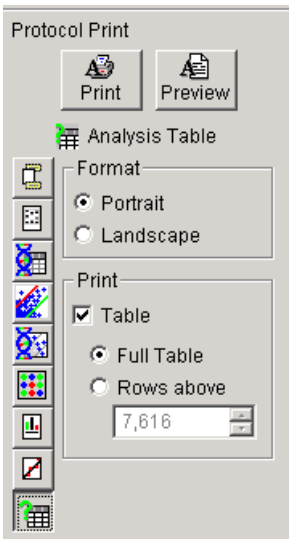



*Figure 2-67 Analysis Table Print Options*

# Print

In AIDA there are two ways for printing your image and evaluation data:

1. Select the **Print** option from the **File** menu, or
2. use the **Protocol Print** section on the **Publishing Layout** toolbox.

The **Print** command of the **File** menu allows you to print your data 'on the fly' without permanently storing the layout/printing settings, whereas the **Protocol Print** panel provides you with a complete set of printing features which includes saving the setting with the data file for reasons of reproducibility.

Selecting the **Print** option from the **File** menu opens the **Print Microarray** dialog, allowing you to configure the printout to contain only the overlays, results and graphs that are currently needed.

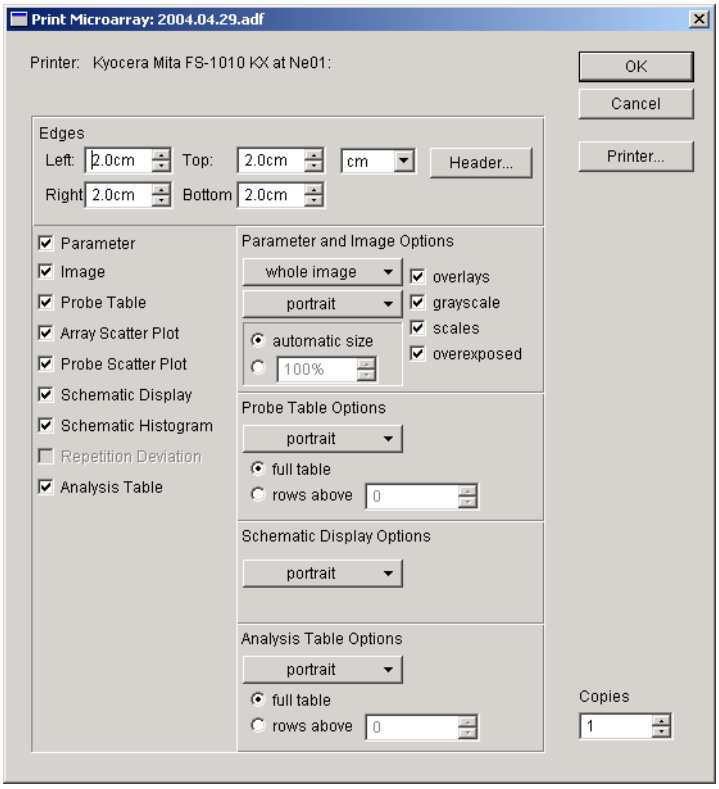



*Figure 2-68 Print Microarray Dialog*

Click the check boxes for the various items to enable or disable them. Select the number of copies, you want to print.

The margins of the printout can be selected in the **Edges** part of the dialog. The **Header** button opens the following dialog:

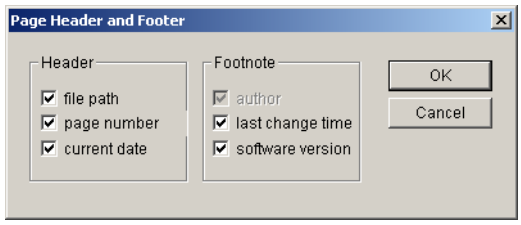



*Figure 2-69 Page Header and Footer Dialog*

Here the **Header** and **Footer** of the report can be configured to contain various image and evaluation specific data.

The **Parameter** (data overlays), the **Image**, **Probe Table**, **Array Scatter Plot**, **Probe Scatter Plot**, **Schematic Display**, **Schematic Histogram**, **Repetition Deviation**, and **Array Analysis Table** information can be selected in the left part of the **Print Microarray** dialog.

In the right part of the dialog (**Parameter and Image Options** section) you can select **Whole Image**, or **Window Content** from the first drop-down menu. Choose **Landscape** or **Portrait** format from the second drop-down menu. Further, the printing of **Overlays**, **Grayscales**, **Scales**, and **Overexposed** can be enabled by checking the respective options. The printout can be configured to automatically fit to the page, or the relative size of the image representation can be selected (in %). In the **Analysis Table Options** and **Probe Table Options** sections you can specify to print the entire table (**full table**) or just a part of it (**rows above**) as well as the paper orientation (**landscape** or **portrait**). The paper orientation can also be specified for the **Schematic Display** output using the drop-down menu in the **Schematic Display Options** section.

# Preview

As for printing, in AIDA there are two ways of previewing pages before they are actually printed:

1. Using the **Print Preview** option of the **File** menu, or
2. using the **Preview** button on the **Publishing Layout** toolbox.

The **Print Preview** option of the **File** menu opens the **Preview** dialog, which allows you to specify the layout settings for the preview. The options presented here are the same as in the **Print Microarray** dialog and those selectable from the various sections of the **Publishing Layout** toolbox. However, they are not stored with the data file.

The **Preview** option of the **Publishing Layout** toolbox allows you to preview all pages as configured in the **Publishing Layout** toolbox before they are actually printed. Clicking the **Preview** button opens the **Preview** window directly, since all layout setting are already made through the **Protocol Print** panel.

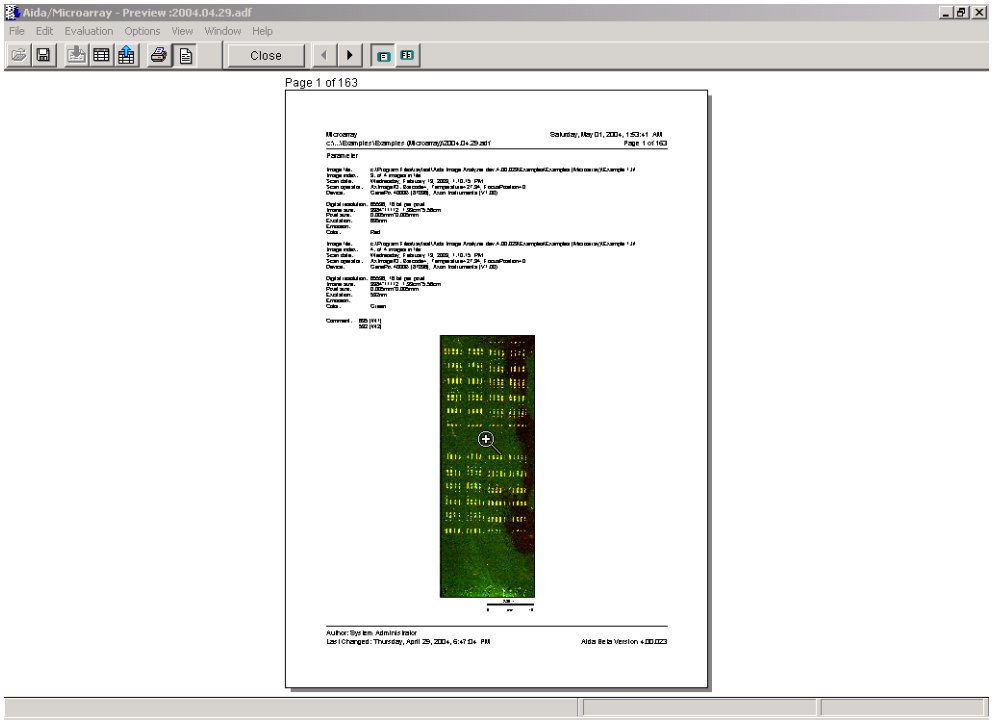



*Figure 2-70 Preview Example*

# 2

# Export and Import

In AIDA Microarray, array definition as well as the content of all windows can be exported to a specific file.

## Exporting Templates

You can *save* your own array definition including all evaluation parameters (for example background definition parameters, reference definition parameters, column selection) to a template file and import it to any other image.

**To export your own array definition:**

1. Choose **File** > **Export** > **Template**.
2. In the **Export Template** dialog that appears enter a name for the export file and confirm the action by clicking **Save**.

   Or:

1. Click the **Export Template** button on the toolbar to open the **Export Template** dialog.
2. Enter a name for the export file in the **File name** text box and complete the export action by clicking **Save**.

## Exporting Array Result Tables

**To export the Array Result Table:**

1. Choose **File** > **Export** > **Array Result Table**.
2. In the **Array Result Table Export** dialog that appears enter a name for the export file.
3. Click **Save** to confirm the action.

The **Array Result Table Export Options** dialog appears, allowing you to set further option for the export of the array results.

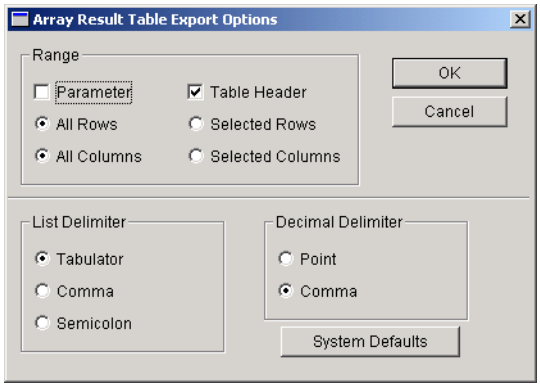



*Figure 2-71 Array Result Table Export Options Dialog*

You can specify the **Range** of the exported rows and columns as well as the **List Delimiter** and **Decimal Delimiter** in the respective panels of the dialog.

4 Complete the action by clicking the **OK** button.

The data can be saved to a simple ASCII (text) or MS Excel file.

## Exporting Probe Tables

**To export the Probe Table:**

1 Choose **File** > **Export** > **Probe Table**.

2 In the **Probe Table Export** dialog that appears enter a name for the export file.

3 Click **Save** to confirm the action.

The **Probe Table Export Options** dialog appears, allowing you to set further option for the export of the array results.

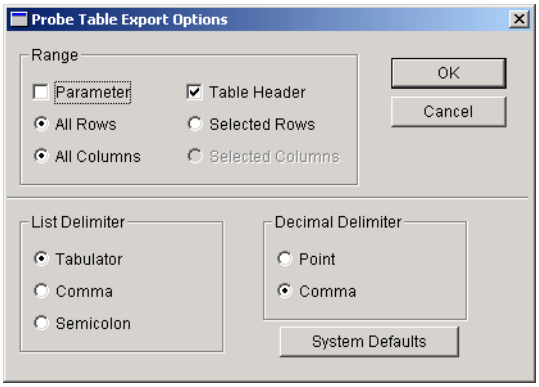



*Figure 2-72 Probe Table Export Options Dialog*

You can specify the **Range** of the exported rows and columns as well as the **List Delimiter** and **Decimal Delimiter** in the respective panels of the dialog.

4 Complete the action by clicking the **OK** button.

The data can be saved to a simple ASCII (text) or MS Excel file.

## Exporting Array Scatter Plots

**To export Array Scatter Plots:**

1 Choose **File** > **Export** > **Array Scatter Plot**.

2 In the **Array Scatter Plot Export** dialog that appears enter a name for the export file.

3 Complete the export action by clicking the **Save** button.

The data are saved to a file in the Windows Metafile or Windows Enhanced Metafile format, depending on your file type selection.

## Exporting Probe Scatter Plots

**To export Probe Scatter Plots:**

1. Choose **File** > **Export** > **Probe Scatter Plot**.
2. In the **Probe Scatter Plot Export** dialog that appears enter a name for the export file.
3. Complete the export action by clicking the **Save** button.

   The data are saved to a file in the Windows Metafile or Windows Enhanced Metafile format, depending on your file type selection.

## Exporting Schematic Display

**To export the Schematic Display:**

1. Choose **File** > **Export** > **Schematic Display**.
2. In the **Schematic Display Export** dialog that appears enter a name for the export file.
3. Complete the export action by clicking the **Save** button.

   The data are saved to a file in the Windows Metafile or Windows Enhanced Metafile format, depending on your file type selection.

## Exporting Schematic Histogram

**To export the Schematic Histogram:**

1. Choose **File** > **Export** > **Schematic Histogram**.
2. In the **Schematic Histogram Export** dialog that appears enter a name for the export file.
3. Complete the export action by clicking the **Save** button.

   The data are saved to a file in the Windows Metafile or Windows Enhanced Metafile format, depending on your file type selection.

# Exporting Analysis Table

**To export the Analysis Table:**

1. Choose **File** > **Export** > **Analysis Table**.
2. In the **Analysis Table Export** dialog that appears enter a name for the export file.
3. Click **Save** to confirm the export.

   The **Analysis Table Export Options** dialog appears, allowing you to set further option for the export of the analysis table.

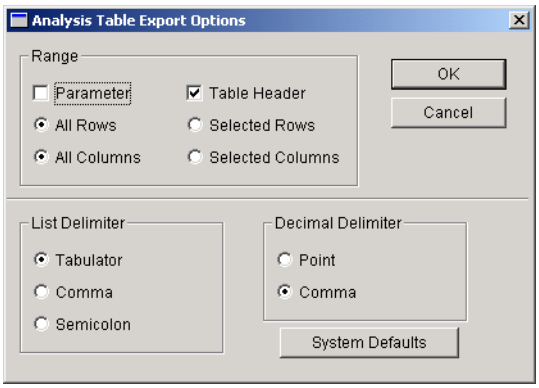



*Figure 2-73 Analysis Table Export Options Dialog*

   You can specify the **Range** of the exported rows and columns as well as the **List Delimiter** and **Decimal Delimiter** in the respective panels of the dialog.

4. Complete the action by clicking the **OK** button.

   The data can be saved to a simple ASCII (text) or MS Excel file.

## Automatic Export of the Array Tables

AIDA's **Automatic Export** option allows you to export all array tables (**Array Result Table**, **Analysis Table**, **Probe Table**) automatically when you save an evaluation.

You can specify preferences for the range of the table columns to be automatically exported as well as export settings, which are independent from the current work configurations of the table(s).

The naming of the export files follows the scheme "*Evaluation Name*.Array Result Table.txt", "*Evaluation Name*.Analysis Table.txt" or "*Evaluation Name*.Probe Table.txt", that is, if the name of the evaluation file is `example.adf`, the Array Result export file has the name `example.Array Result Table.txt`, the Analysis Table export file is named `example.Array Result Table.txt`and the Probe Table export file `example.Probe Table.txt`. Existing export files are overwritten without prompting the user. However, a backup file of the previous export file version is created.

**Automatic Export** always exports the entire table. Restricting the export to selected rows is not possible.

The enabling of the automatic export option as well as its settings are evaluation-specific attributes, which are valid until they are explicitly changed using the **Preferences** or **Automatic Export** options of the **Options** menu or by importing an AIDA template. If you create a new evaluation, the current default configuration for **Automatic Export** is applied and will be saved with the evaluation data in the `adf.` file. The configuration remains valid unless it is explicitly changed by the user.

The automatic export settings are timestamped internally. In addition you can add a name and an ID, which are included in the Parameter pane of the export file together with the internal time stamp.

1 Choose **Options** > **Automatic Export**.

The **Automatic Export Setting** dialog appears.

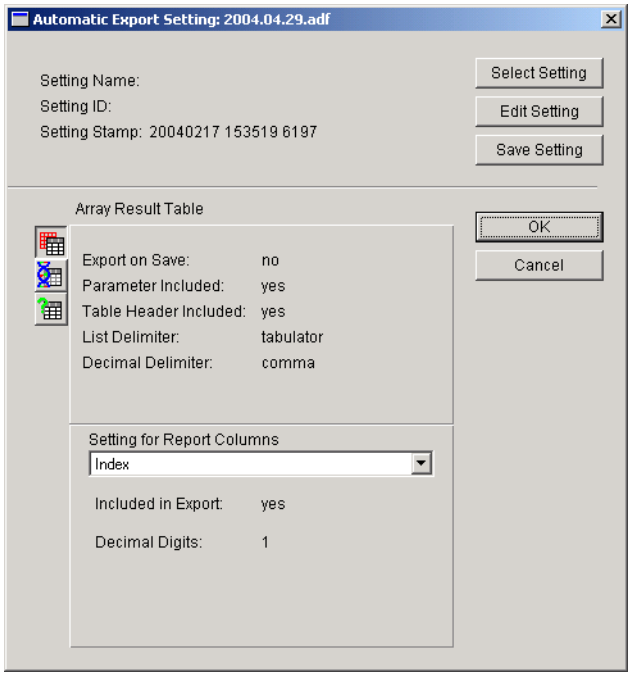



*Figure 2-74 Automatic Export Setting Dialog*

The top pane of the dialog shows the current settings parameters, whereas the second displays the current settings for the automatic export of the Array Result Table/Analysis Table/Probe Table. Click on the toolbar to switch between the settings pages.

2 Click **Edit Settings**.

The **Edit Automatic Export Setting** dialog opens.

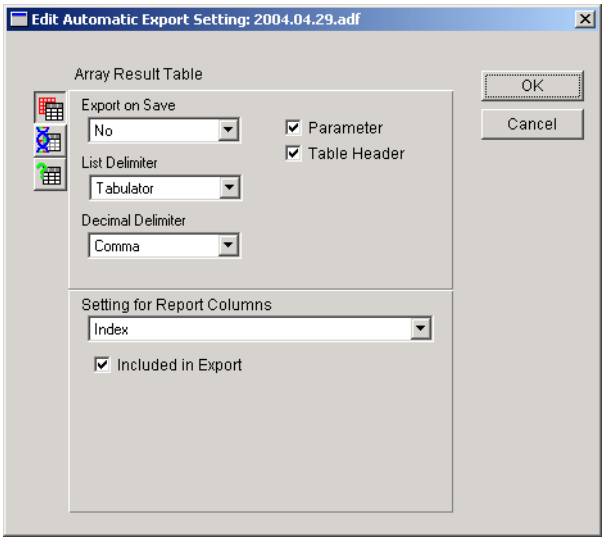



*Figure 2-75 Edit Automatic Export Setting Dialog*

The dialog provides you with the following option:

- **Export on Save: Automatic** enables the automatic export and generates the export filename automatically from the evaluation filename. **No** disables the automatic export. If you select **Prompt for**, you will be prompted for an export data filename, when you save an evaluation.
- **Parameter:** enables/disables the export of the table's parameter pane.
- **Table Header:** enables/disables the export of the table header.
- **List Delimiter:** allows you to select a delimiter for the columns
- **Decimal Delimiter:** allows you to select a decimal delimiter format.
- **Settings for Report Columns:** select columns subsequently and click to select or deselect the **Included in Export** option to include/exclude the respective column. Depending on the column you selected, you can specify additional settings (in this example: the **Decimal Digits**) for the exported values.

3 Select the appropriate options and click **OK** to complete the action.

### Saving Automatic Export Settings:

1 On the **Automatic Export Setting** dialog, click **Save Setting**.

The **Save Automatic Export Setting** dialog opens.

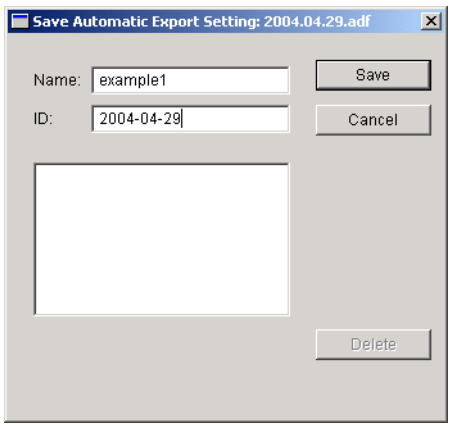



*Figure 2-76 Save Automatic Export Setting Dialog*

2 Type a unique name and an ID for the settings and click **Save** to confirm the action.

### Selecting Automatic Export Settings

1 On the **Automatic Export Setting** dialog, click **Select Setting**.

The **Select Automatic Export Setting** dialog appears.

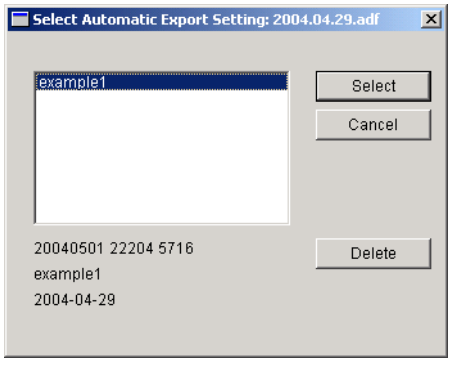



*Figure 2-77 Select Automatic Export Setting Dialog*

2 Select a settings set and click **Select** to complete the action.

**To delete a settings set:**

1. Select the set you want to delete in the list of the **Select Automatic Export Setting** dialog.
2. Click **Delete**.

## Importing Templates

Array templates are stored with the program and can be imported using the **Import** option of the **File** menu or by clicking the **Import Template** button:

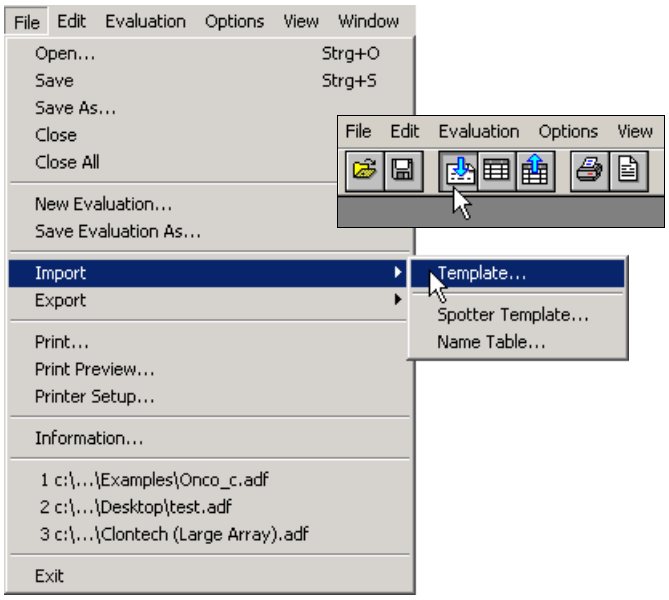



*Figure 2-78 Importing Templates*

# Importing GAL Templates

GAL (GenePix Array List) templates are generated by many micro-array spotters. A GAL template file contains the geometrical/structural as well as probe information of the array. AIDA Microarray directly imports the information of the GAL template file and uses this information to automatically build its internal array geometry and name table.

### To import a GAL template

1 On the **File** menu, click the **Import** menu item and then select **Spotter Template** from the cascading menu.

The **Spotter Template Import** dialog appears.

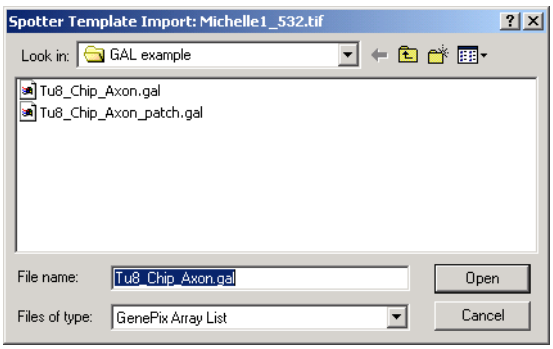



*Figure 2-79 Spotter Template Import Dialog*

2 Select a GAL file and click **Open**.

AIDA Microarray creates an array overlay and a name table from the imported data.

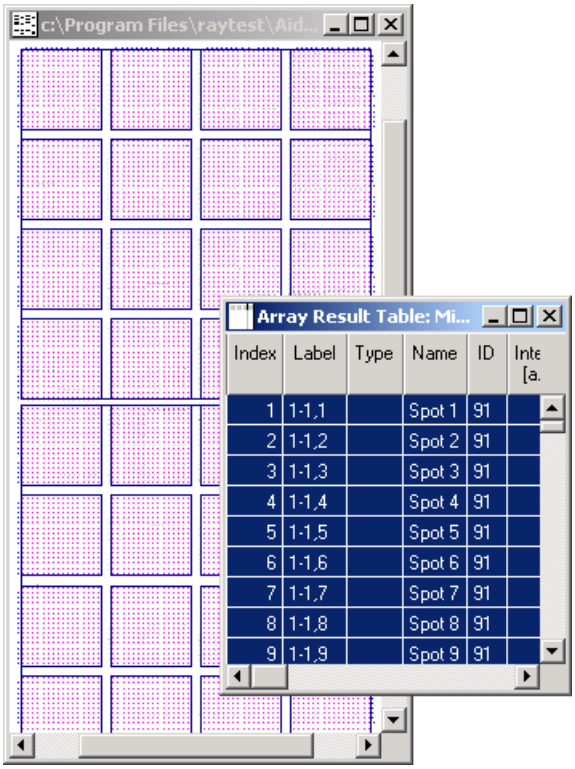



*Figure 2-80 Array Overlay And Nametable Created From Imported Data*

## Importing Name Tables

To import nametables (names and ID numbers of samples) to the **Array Result Table**, use the **Import** option of the **File** menu or alternatively the **Name Table Import** option of the **Array Definition** or **Array Measurement** toolbox.

The **Name Table Import** dialog appears allowing you to specify the options for the name table import.The table has to contain the same labeling as defined in the array (for details, see the Import of Name Tables section below).

# 2

# Performing Microarray Evaluations 3

## Standard Evaluation Procedure

With a few simple steps precise data from array experiments can be generated using the automatic evaluation feature provided by AIDA Microarray. The steps of the standard evaluation procedure described below requires that an suitable array template already exists. This can be either an previously saved user-defined template or one of the built-in pre-defined templates shipped with AIDA Microarray. If there is no suitable template available, you either have to define a new one from scratch (see the “Defining a New Template” section for details) or to modify an existing template (see the “Manual Alignment Adjustments” section for details).

## Step 1: Loading the Image File

**To load a multi-channel image file (FUJI SET file or multi-channel TIFF file):**

1 Choose **File** > **Open**.

2 Select the image, you want to load from the **AIDA Data** dialog and click **Open**.

3 In the **Open Multi Channel Data Set** dialog, check the channels to be loaded.

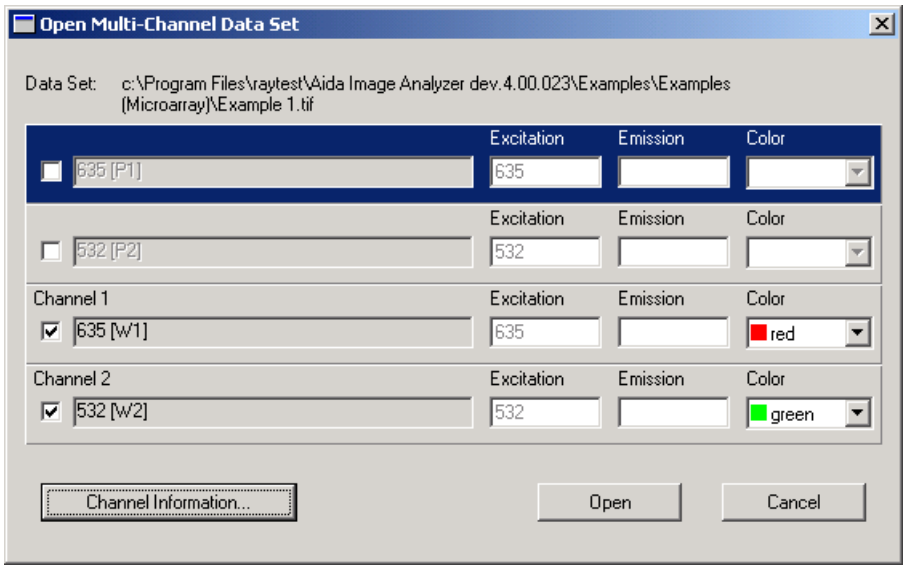



*Figure 3-1 Open Multi Channel Data Set Dialog*

4 Choose the display color for each channel from the respective **Color** menus.

5 (Optional:) Enter the missing information (for example, emission filter data) in the corresponing fields.

6 Click **Open**.

**To open one or more single-channel image files:**

1 Choose **File** > **Open**.

2 Select the image, you want to load from the **AIDA Data** dialog and click **Open**.

3 In the **Compose Multi Channel Data Set** dialog, click **Add Channel** to add additional single-channel images to the data set resulting in a multi-channel data set.

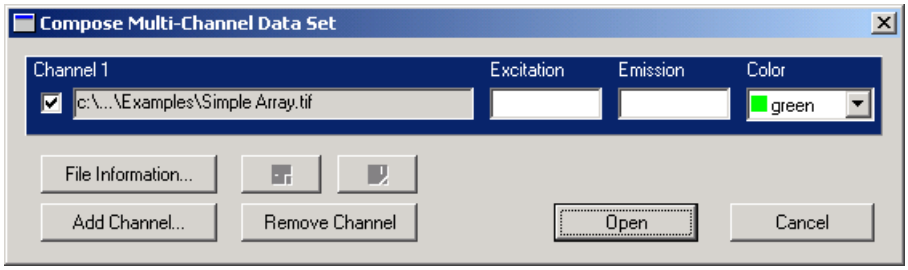



*Figure 3-2 Compose Multi Channel Data Set Dialog*

4 Choose the display color for each channel from the respective **Color** menus.

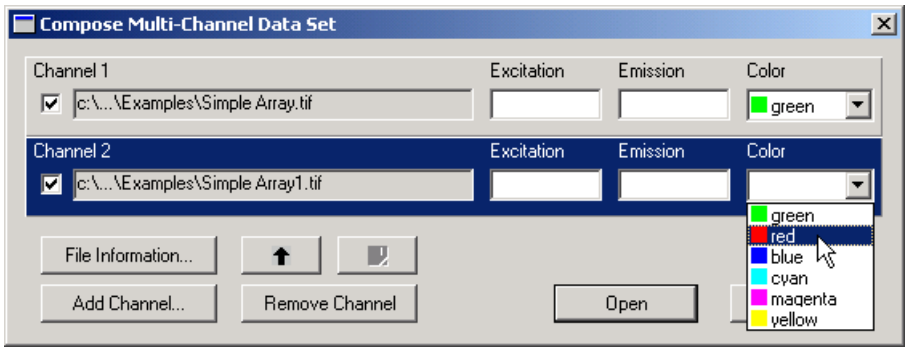



*Figure 3-3 Choosing Display Color for Channel*

5 (Optional:) Enter the missing information (for example, emission filter data) in the corresponing fields.

6 Click **Open**.

## Step 2: Loading the Template

In AIDA Microarray you can load either one of the built-in pre-defined array templates shipped with the application or import an previously saved user-defined template.

**To load a predefined array template:**

1 Click the **Import Array Template** button in the **Template** section of the **Array Measurement** toolbox.

This changes the appearance of the **Template** section, allowing you to select array templates from the template library.

2 Select an array template and click **OK**.

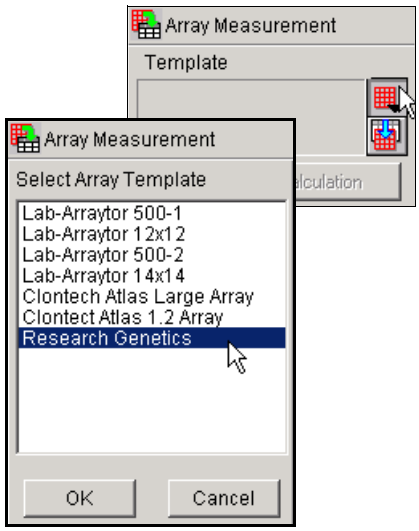



*Figure 3-4 Selecting Array Template*

The resulting array overlay appears in red indicating the array overlay's EDIT status. In addition, the appearance of the toolbox section changes again, revealing spin boxes for fine-tuning the adjustment of the array template in terms of array overlay x- and y-offset, image and array resolution as well as spot diameter (see “Step 3: Adjusting the Template to the Image” for details).

**To load a previously saved user-defined array template:**

1 Choose **File** > **Import** > **Template**, or click the **Import Template** button on the main toolbar.

2 Choose the template file, you want to import from the file list in the **Import Template** dialog.

Now all data settings and overlays from the array template file are imported to the image data. The overlays in the array template are imported in activated and changeable mode. You can adjust their position and resize or rotate these overlays before they are applied to the image (see “Step 3: Adjusting the Template to the Image” for detail).

## Step 3: Adjusting the Template to the Image

**To adjust an imported template:**

1 In the **Array Template** panel of the **Array Measurement** toolbox, which appears directly after loading a template, enter the appropriate values in the spin boxes, AND/OR edit the template overlay directly by dragging the overlay to the desired position.

The **Array Template** panel contains spin boxes for fine-tuning the adjustment of the array template in terms of array overlay x- and y-offset, image and array resolution as well as spot diameter.

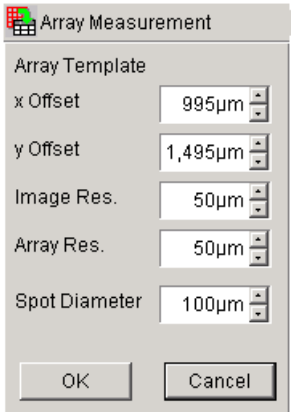



*Figure 3-5 Array Template Adjustment Controls*

Setting the **Image Res.** or **Array Res.** parameters can be necessary, if the scanning device used to acquire the image didn't embed the pixel resolution date for the image in the image file. In this case, the value in the template are interpreted as pixel values. This can lead to misjudgments. If you know the resolution of the image, you can enter it in the Image Res. spin box to circumvent this problem.

In addition, you can adjust the spot diameter, if necessary, using the respective spin box.

2 After performing the necessary modification to the overlay, click **OK** or alternatively right-click on the overlay to confirm the changes and switch the status of the overlay to ACTIVATED.

The message box to the left of the **Import Array Template** button shows the name of the imported template.

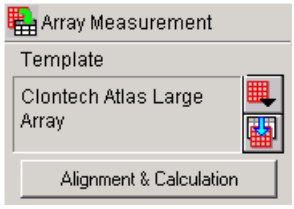



Figure 3-6 Template Import Completed

## Step 4: Starting Automatic Alignment and Calculation

**To start the automatic alignment and calculation process:**

**3** On the **Array Measurement** tool, click **Alignment & Calculation**.

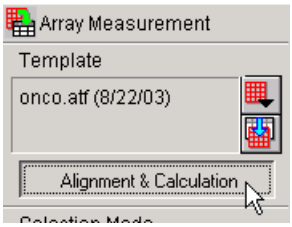



*Figure 3-7 Starting Automatic Alignment and Calculation Process*

The alignment process is performed in two steps: the positioning of the overlay and the separation of the measurement signal from the background signal (so called *segmentation*). **Alignment & Calculation** performs the steps in sequence.

Positioning and segmentation of the spots is done for all channel simultanously. For internal processing purpose, the values of each channel are weighted using the standard deviation of the intensity values of the background pixels. This allows the comparion of the channel data.

During the positioning process, the blocks of the array are aligned according to the maximum intensity value of the sum of the spots in each block. Then each spot in the block is moved to a position with minimal circulare intensity fluctuations.

After the positioning process is completed, outlier spots are determined and then included in the grid structure.

If **Adaptive Diameter Segmentation** is selected, the diamater of each spot is determined by minimizing the distance between signal and spot center and the distance between background and the spot's peripherial region.

The automatic alignment and calculation process matches the position of the defined array spots with the position of the real dots in the array and performs all remaining measurement calculations (background subtraction and so on).

The intensity values of all spots, which have been aligned and integrated can be corrected for background. As described in the respective section of this manual, you can select the appropriate background subtraction method from the drop-down menu of the **Settings** section of the **Array Definition** toolbox. The subtraction the background values from each spot is performed automatically by the **Alignment & Calculation** procedure.

The procedure tests guide dots to be in maximum, paired dots to have almost equal intensities and empty dots to be in minimum. If no spotting pattern is available or the **use spotting pattern** option is switched off, the spotting matrices are moved to the nearest maximum.

In the second step of the alignment, the program moves each dot above the threshold to the nearest maximum of averaged intensities. The dots below this threshold, or dots where no maximum is found, are set by the program to a position which is calculated from the aligned dot positions.

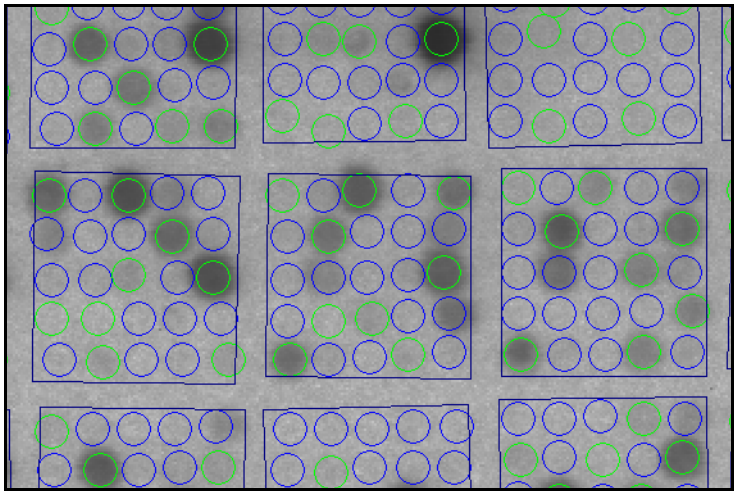


*Figure 3-8 Aligned Array Example*

Spots with at least one overexposed pixel (magenta) are automatically marked as "bad" and tagged with the label "sat." (for "saturated") in the **Quality** column of the result tables.

## Step 5: Saving the Evaluation

After the **Alignment & Calculation** process is completed, save the evaluation with the automatic export option enabled. All measurement data are exported to corresponding files.

**To save the evaluation:**

1. Choose **File** > **Save**, or **File** > **Save New Evaluation As**.
2. In the **Save New Evaluation As** dialog, select a location, enter a file name and click **Save**.

# Manual Alignment Adjustments

In most cases, the automatic alignment process described in the previous section successfully aligns defined array spots and real dots. But sometimes it fails, due to irregular dotting patterns. In this case you might want to adjust the alignment manually. You can do this by using the standard overlay editing features provided by AIDA Microarray.

You can perform the following three modification:

- Adjusting parts of the overlay for better fitting with the image
- Adjusting the position of particular dots
- Adjusting the alignment parameters (using the **Alignment** controls of the **Array Measurement** toolbox)

## Adjusting parts of the overlay for better fitting with the image

**To adjust parts of the overlay:**

1. Select the area of the overlay you want to adjust using the respective selection tool.
2. Double-click on the selected overlay area to switch to EDITING mode.
3. Edit the overlay's fields, block and/or spot saccording to your needs.

   In AIDA Microarray the following three different editing modes are available for array overlays. Toggle between these modes by pressing the Space key:

- **Distorting**: Drag one of the squared handles attached to each of the four corner of the array overlay in any direction while holding down the mouse button. This introduces trapeze-shaped distortions.

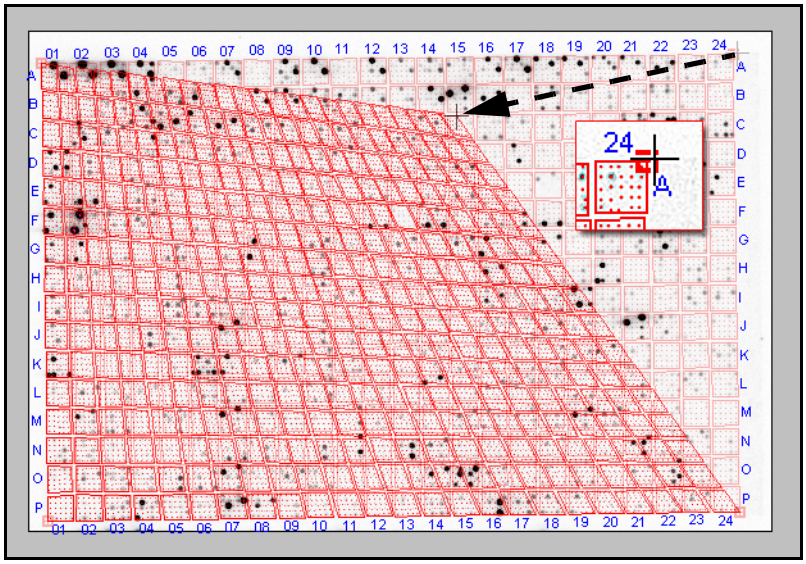



*Figure 3-9 Distorting Array Overlay*

- **Stretching and Compressing**: After switching to this editing mode, the array overlay appears with squared handles attached to each corner *and edge*. Dragging a corner handle stretches or compresses the array along the longitudinal *and* traverse axis simultaneously.

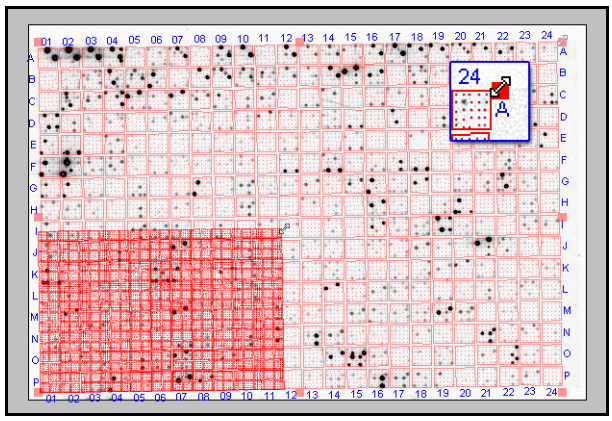



*Figure 3-10 Stretching/Compressing Array Overlay (X-/Y-Axis)*

Dragging an edge handle stretches or compresses the array overlay in one direction only.

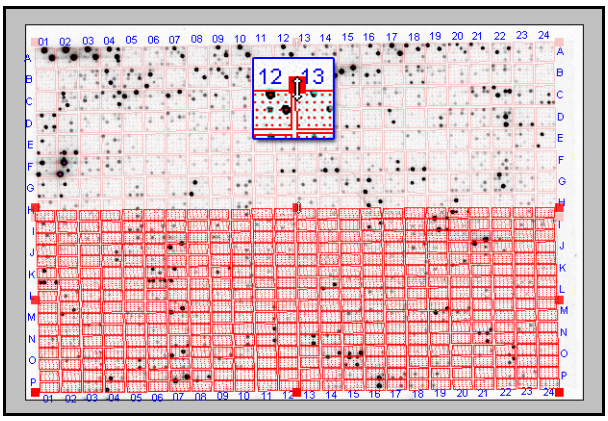



*Figure 3-11 Stretching/Compressing Array Overlay (Y-Axis Only)*

- **Rotating Array Overlays:** Switching to this mode changes the six squared handles of the previous mode to circle-shaped ones. Dragging a corner circle rotates the array overlay around the opposite corner.

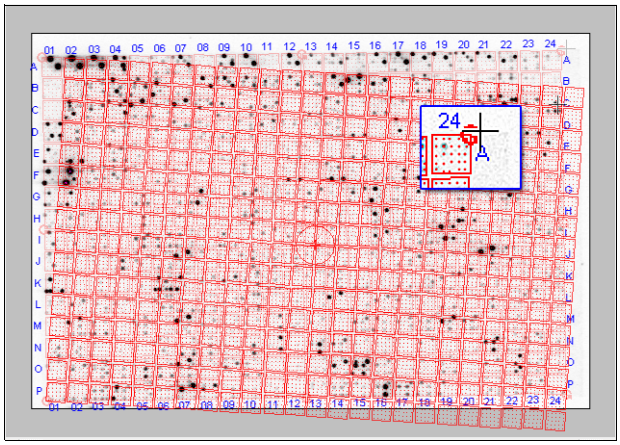



*Figure 3-12 Rotating Array Overlay Around the Lower Left-Hand Corner*

Dragging a edge circle rotates the overlay around its center.

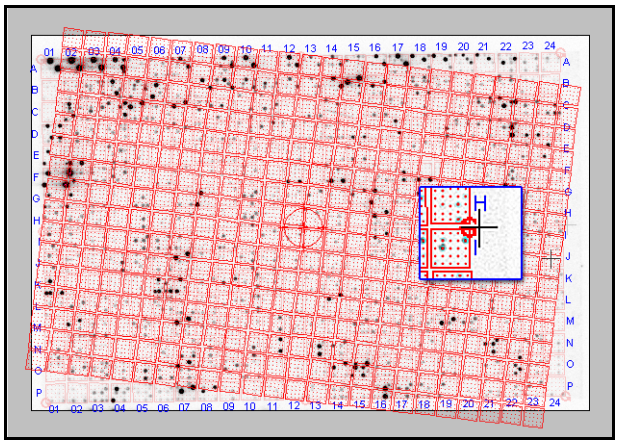


*Figure 3-13 Rotating Array Overlay Around Center*

In addition, you can move the center of the array overlay using the mouse, thereby moving its pivot point.

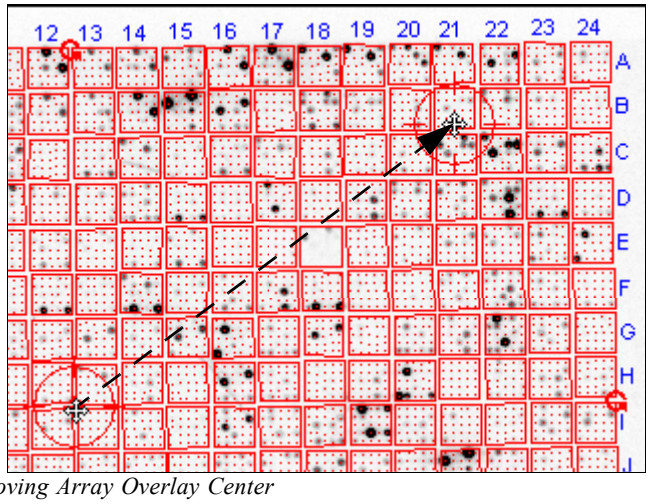


*Figure 3-14 Moving Array Overlay Center*

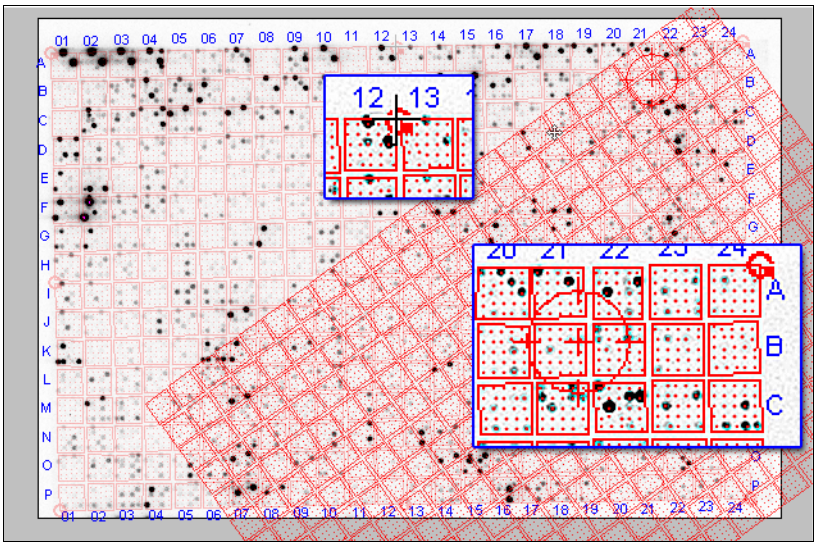



*Figure 3-15 Rotating Array Overlay Around Moved Center*

Since the array overlay center's pivot point function is limited to rotation task performed by dragging handles located at the edges of the overlay, moving the center does not affect rotation tasks performed by dragging corner handles:

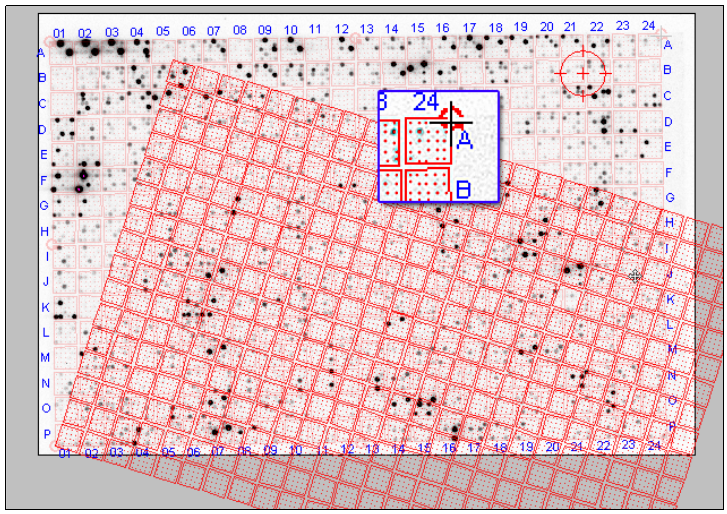



*Figure 3-16 Rotating Array Overlay Using Corner Handle (Center Moved)*

Confirm your modification by clicking the right mouse button or alternatively pressing the Return key.

4 Right-click to apply the changes.

## Adjusting the position of particular dots

In addition to editing the fields' and blocks' overlay geometry and position, you can adjust the position of particular spots and/or change their diameter:

**To adjust the position of a spot:**

1 Double-click the spot, which you want to reposition to switch to EDITING mode.

2 Drag the spot overlay to the desired location.

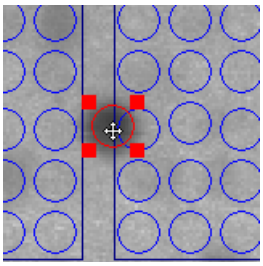


*Figure 3-17 Adjusting Spot Position*

3 Right-click to apply the changes.

**To adjust the diameter of a spot:**

1 Double-click the spot, which you want to reposition to switch to EDITING mode.

2 Drag one of the edge handles of the overlay to increase or decrease its diameter.

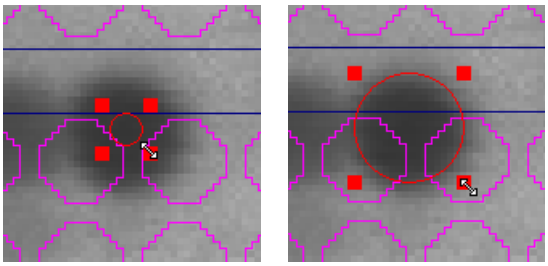


*Figure 3-18 Adjusting Spot Diameter*

3 Right-click to apply the changes.

# 3

## Adjusting the alignment parameters

**To control the alignment process:**

1. On the **Alignment** panel of the **Array Measurement** toolbox, check the appropriate options for the alignment process: You can choose to adjust the position the overlay automatically (**Auto Positioning**) and/or to separate measurement signals from background signals (**Segmentation**). In addition, you can choose to apply the alignment process to selected areas only (**restrict to selection**).
2. Click **Align** on the **Alignment** panel of the **Array Measurement** toolbox to start the alignment process.
3. Click **Final Calculation** to perform all remaining calculations.

# Defining a New Template

If a completely new evaluation is necessary, you can either import an appropriate array template, which you have previously defined and stored or which you select from the library of predefined templates shipped with the Microarray module, or else create an array overlay from scratch using the **Grid Definition** controls of the **Array Definition** toolbox.

The definition process consists of up to three steps, according to the number of possible hierarchic array levels.

## Array Levels

Array overlays consist of up to three levels, which can be selected for individual editing.

The *simplest array structure* inherits only the spotting matrix. It consists of a first level with 1x1 fields and a third level comprised of 20x18 dots.

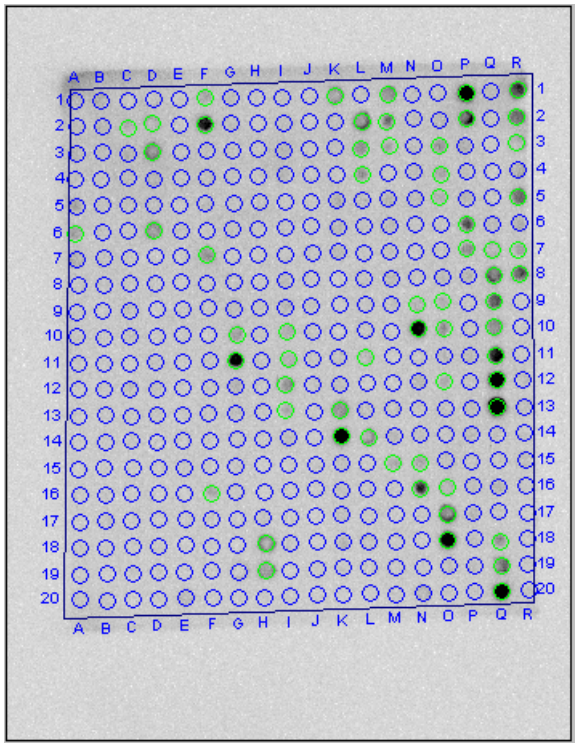



*Figure 3-19 Simple Array Structure Example*

The most common structure consists out of two levels, containing the surrounding frame with the blocks (16x24 fields) and the spotting matrix (5x5 spots) in each block respectively.

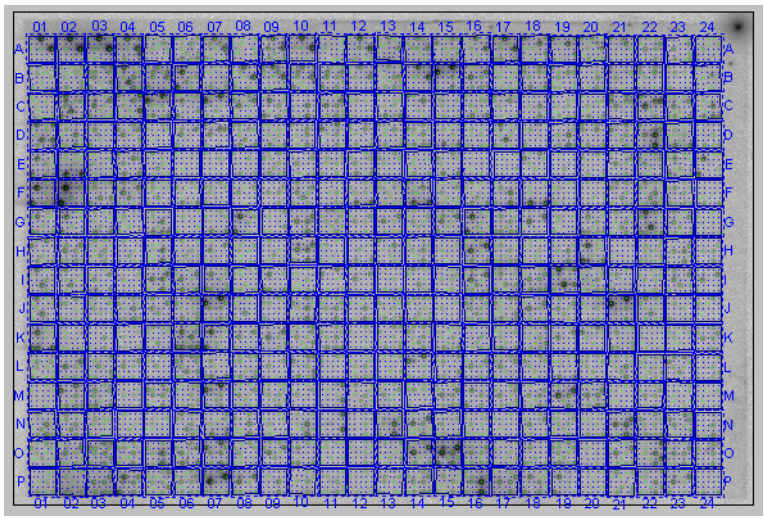



*Figure 3-20 Most Common Array Structure*

Large arrays can have up to three array levels. In the example below, the array is comprised of three levels: a first level containing 3x2 fields, a second level with 16x24 fields, and a third (spotting) level with 4x4 dots.

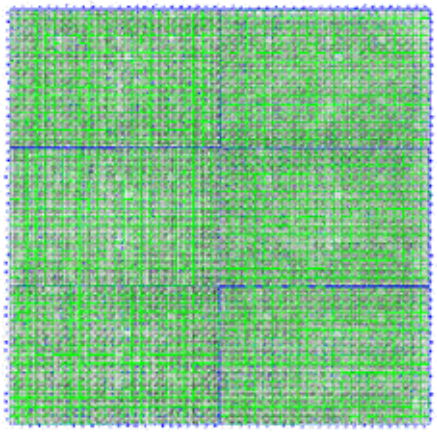


*Figure 3-21 Large Array Structure*

# Defining a Field Matrix (Array)

To define the first array level (the surrounding frame, overall mesh structure, and repetition pattern), select the **New Evaluation** item from the **File** menu. All previous array overlays are cleared.

## Array Definition Settings

**To define the geometry and labeling of the field matrix:**

1 Click the **Edit Array Fields** button on the **Grid Definition** panel of the **Array Definition** toolbox.

The appearance of the **Grid Definition** panel changes to reveal controls for specifying the number of columns (**Columns**) and rows (**Rows**), spacing between columns (**Y Spacing**) and rows (**X Spacing**) as well as their respective labeling.

In addition, the **Repetition Pattern Definition** section of the pane allows you to specify repeated fields in the array.

2 To divide the total array area into sub sections, enter appropriate values in the **Rows** and **Columns** spin boxes.

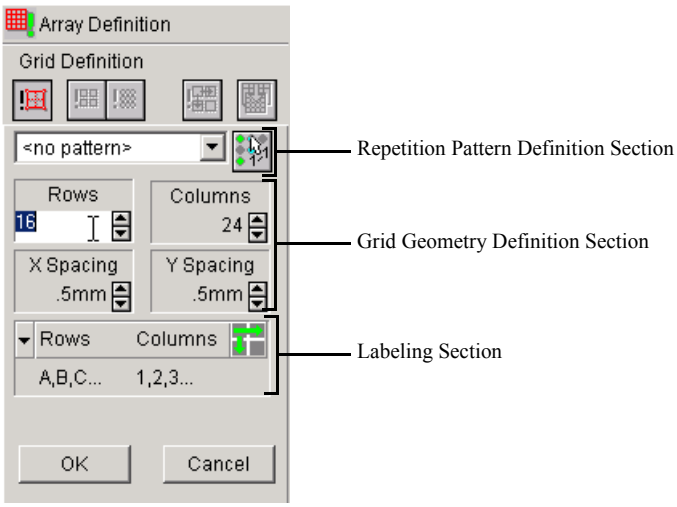



*Figure 3-22 Grid Definition Panel*

3 To introduce spacing between sub sections, enter appropriate length unit values in the **X Spacing** and **Y Spacing** spin boxes respectively.

The defined sub areas will be separated by the distances specified.

4 Click the downward arrow in the left corner of the Labeling section directly below the **X Spacing** and **Y Spacing** spin boxes to determine whether to apply labels to the sub areas of the array and how the labeling is performed.

A drop-down menu appears, allowing you to choose between the labeling types **No Labels**, **Rows by Columns**, **Columns by Rows**, **Sequential** and **Repeat Pattern**.

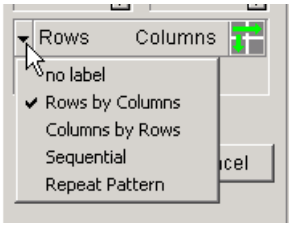



*Figure 3-23 Labeling Type Drop-Down Menu*

5 To define labels for rows and/or columns, click on the respective heading of the Labeling section.

6 Pick the appropriate label (by numbers, uppercase or lowercase letters) for your columns and/or rows from the drop-down menu, that opens.

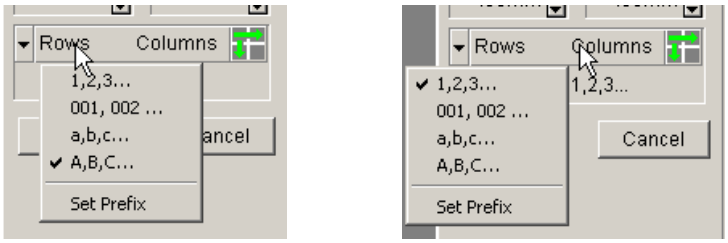



*Figure 3-24 Selecting Labels for Rows and Columns*

In addition, you can set a prefix (**Set Prefix** option) to separate the rows and columns by a special character, or to separate this label level from the labels of the upper array level, in order to avoid confusion in the connected label for spots (if you are using hierarchical labels for the spots).

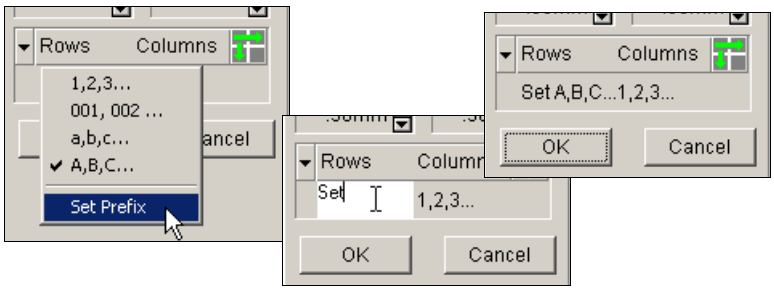



*Figure 3-25 Setting Prefix*

**7** To specify the appropriate label ordering, click on the icon in the right corner of the panel's heading

**8** From the drop-down menu, that appears, select the appropriate entry.

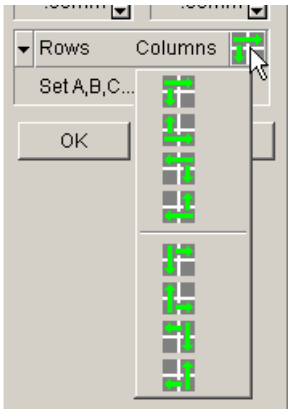



*Figure 3-26 Setting Label Ordering*

## Specifying Field Repetition Pattern

Repetitions are spots from the same probe. They can be defined as repeated spots in a block, repeated blocks in a field and repeated fields in the array. In AIDA Microarray any number of repetitions on any level are possible.

**To add a new field repetition pattern**

1 Click the **Pattern Definition** button on the **Repetition Pattern Definition** section.

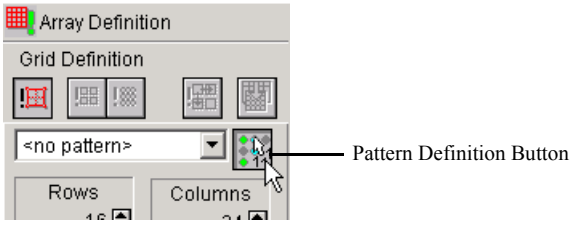



*Figure 3-27 Repetition Pattern Definition Section – Selecting Pattern Definition Button*

The **Select Field Pattern** dialog appears.

2 Click the **Add** button.

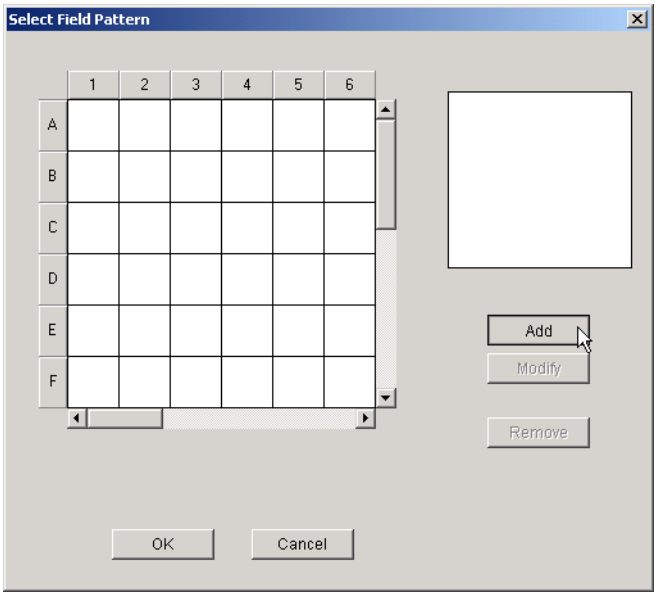



*Figure 3-28 Adding Field Pattern*

The **Add Field Pattern** dialog opens.

**3** Specify the repetition factor, index and increment count using the **repeated**, **index** and **increase** spin boxes respectively.

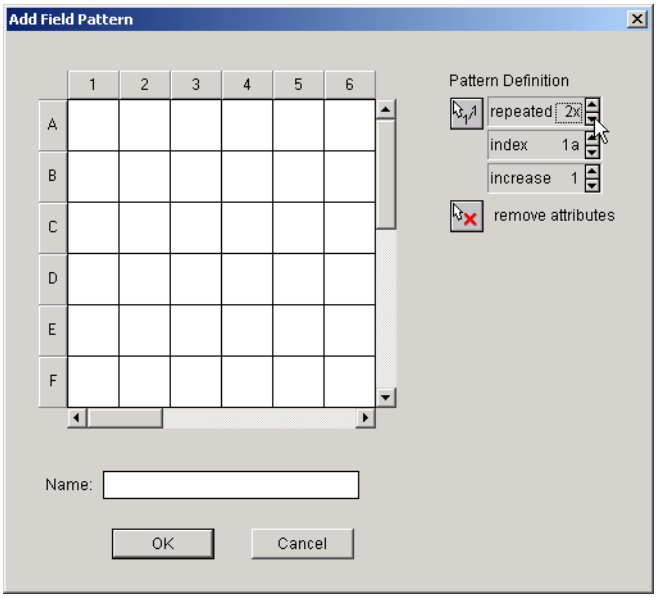



*Figure 3-29 Specifying New Field Repetition Pattern*

**4** To set the repetition positions, click the button next to the **repeated** spin box and then subsequently click in the fields of the schematic array structure displayed.

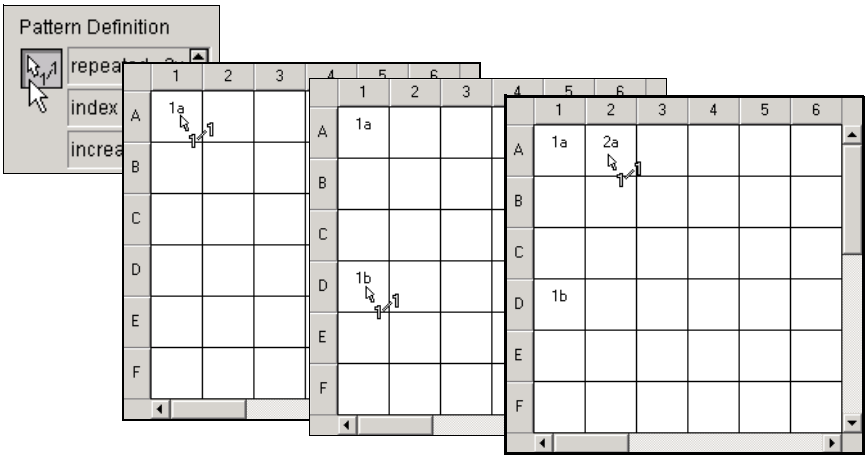



*Figure 3-30 Setting Repetitions*

5 To complete the pattern definition process, enter a name for the pattern in the **Name** field of the **Add Field Pattern** dialog and click **OK**.

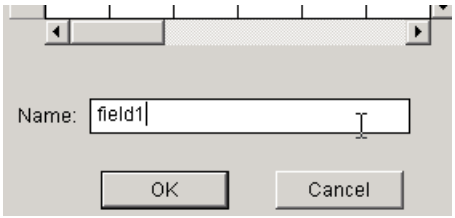



*Figure 3-31 Entering a pattern name*

**To remove repetition attributes from fields**

1 On the **Add Field Pattern** dialog, click the **remove attributes** button.

2 Click in the field, whose repetition attribute you want to remove.

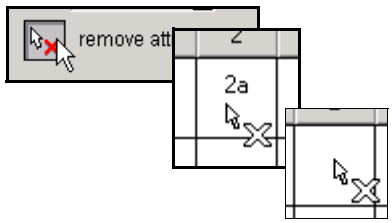



*Figure 3-32 Removing repetition attributes from fields*

**To modify an existing repetition pattern**

1 Click the **Pattern Definition** button on the **Repetition Pattern Definition** section.

2 On the **Select Field** dialog, select a pattern from the list displayed above the push button controls.

3 Click **Modify**.

The **Modify Field Pattern** dialog appears, allowing you to change the pattern definition.

## Defining Border Lines

After you completed the definition settings, turn to the next step of array definition, namely the definition of outer border lines.

**To define the outer border lines of the array overlay:**

1 Set the vertical edge of the array by clicking two positions in the **Image** window.

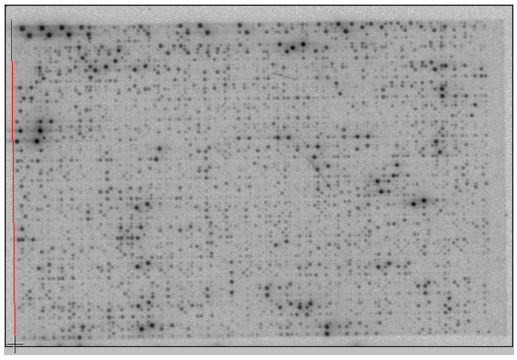


*Figure 3-33 Setting Vertical Edge*

2 Then move the mouse pointer to stretch the frame.

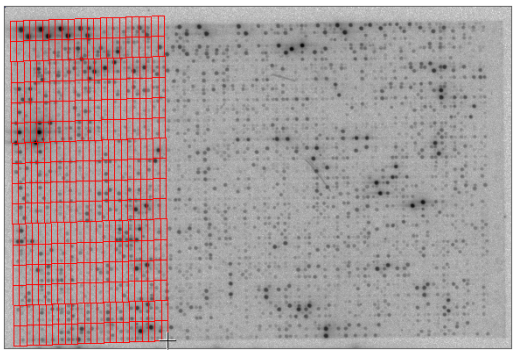


*Figure 3-34 Stretching the Frame*

**3** Finally click the **OK** button in the toolbox to apply the changes.

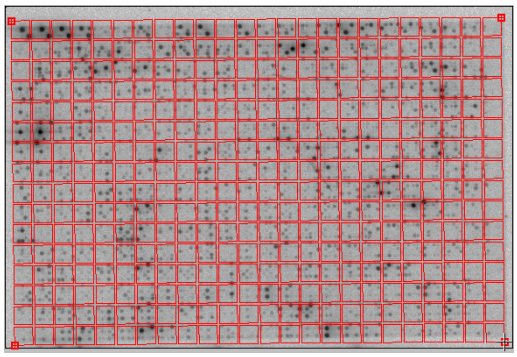


*Figure 3-35 Frame Definition Accomplished*

The new array overlay changes its status from EDIT (red) to ACTIVATED (magenta).

## Defining a Block Matrix (Field)

If necessary you can define a block matrix, which further divides the sub areas determined by the overall array definition (field matrix definition - see above) into smaller sub areas.

On the **Grid Definition** panel of the **Array Definition** toolbox, click the **Edit Array Blocks of selected fields** button to edit the block matrix of selected fields.

*Figure 3-36 Defining Block Matrix*

Again, the **Grid Definition** panel changes its appearance. The options for specifying columns, rows, spacing, labels and repetition pattern provided by the control panel are identical to the ones used to define the overall array (see section above).

## Defining a Spot Matrix (Block)

The spots form the basic structure of an array and are given in rows by columns.

To specify the spot matrix, which determines the number of final spots:

1 Click the **Edit Array Spots of selected blocks** button on the **Grid Definition** panel of the **Array Definition** toolbox.

---

**Note –** Note: The buttons for defining second and third array level will become active only after defining a new overall array overlay (first array level).

---

2 Specify the number of rows and columns in the **Rows** and **Columns** spin boxes respectively.

3 Apply labeling as described for the definition of the first array level above.

4 Define the size of the spots by setting the spot diameter using the **Diameter** spin box.

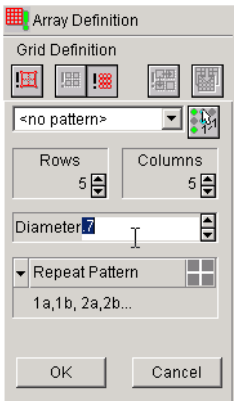



*Figure 3-37 Setting Spot Diameter*

The spot diameter defines the area of intensity measurement of a given spot. It has the same size for a group of simultaneously defined spots.

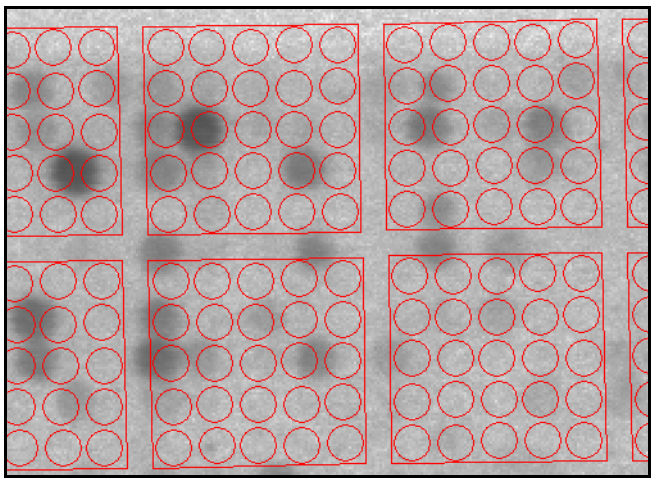


*Figure 3-38 Simultaneous Defined Spots*

## Spotting Pattern

The basic structure of an array frequently contains a defined pattern of spotting, where structures like empty dots or guide dots are located in certain positions and the distribution of double dotted samples is defined.

### Loading Predefined Spotting Pattern

To load predefined spotting pattern, use the **Spotting Pattern** drop-down list on the **Grid Definition** panel (third level) of the **Array Definition** toolbox.

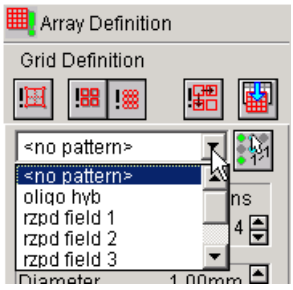



*Figure 3-39 Loading Spotting Pattern via Spotting Pattern Drop-Down List*

## Defining New Spotting Pattern

If you click on the **Pattern Definition** button next to the **Spotting Pattern** drop-down list, the **Select Spotting Pattern** dialog opens, allowing you to define a new spotting pattern or edit/delete a previously saved spotting pattern.

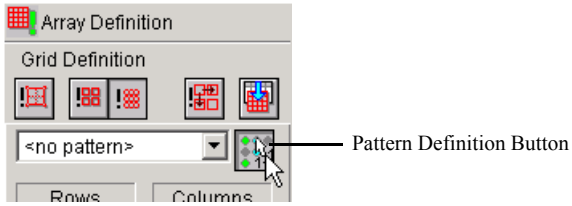



*Figure 3-40 Opening the Spotting Pattern List Dialog to Define a New Pattern*

**To define a new pattern:**

Click **Add**, and then enter a name for the new pattern in the **Name** text box.

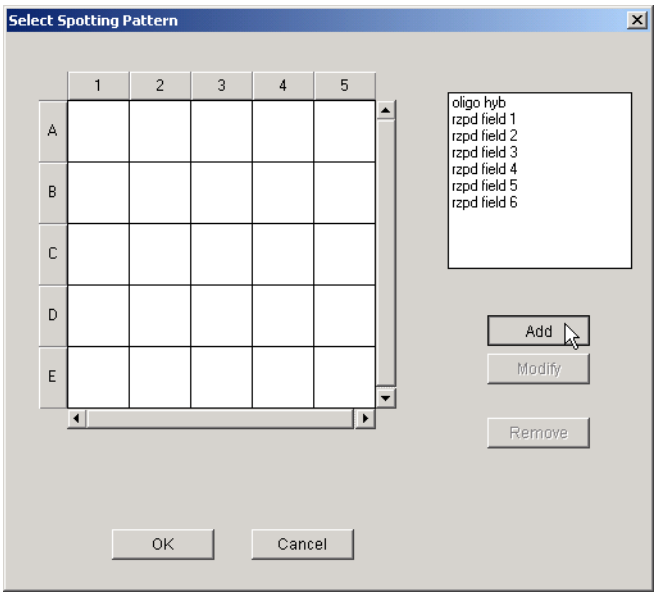



*Figure 3-41 Selecting Spotting Pattern Dialog*

Position empty dots, guide dots, multiple dotting (repetitions), control dots, background dots and reference dots by first clicking the appropriate button in the **Pattern Definition** section and subsequently clicking in the fields of the displayed basic array structure. For

repetitions, any number of repetitions can be set for the array sub-structure using the **repeated *N*x** spin box. Further, to allow easy tracking of the repetitions, you can assign an index and a spotting-related number via the **index** and **increase** spin boxes.

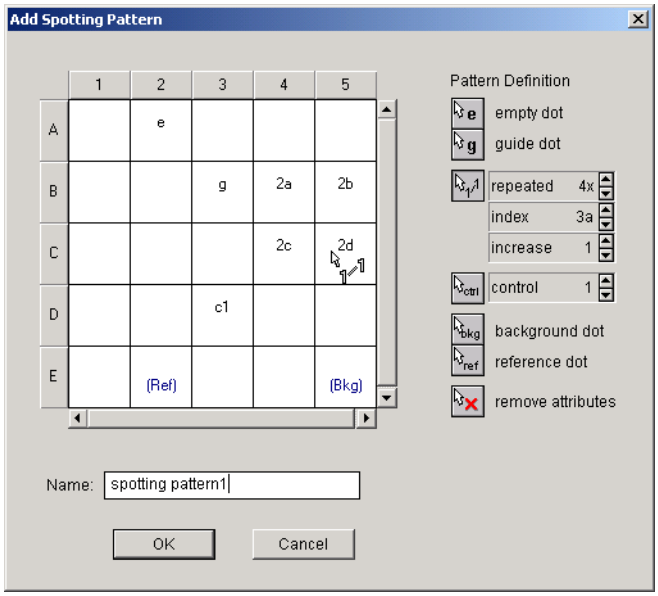



*Figure 3-42 Defining Spotting Pattern*

*Control dots* are spots which are used for quality control (negative controls). Their values are collected and averaged over the entire array. The resulting values are displayed in the **Probe Table**. To ensure the correct collection of the control dot values, always use the same index for corresponding dots.

---

**Note –** If *empty spots* are defined, they can be used for subtraction of a background in the **Background Definition** section of the **Array Definition** toolbox. In addition, you can specify entire background and/or reference dot position patterns for sub grids, which are applied to all sub grids of the array. All flags set by the spotting pattern definition can be overwritten manually using the tools of the **Array Definition** toolbox.
If a *repetition pattern* is given, additional values for the mean of the related spots as well as the absolute and relative deviation of this repeated dots appear in the **Array Result Table**.
*Empty* and *Guide dots* are *not* listed in the **Probe Table**.

---

## Spot Labeling

In addition to the various ways of labeling each level of your array (see above) AIDA Microarray provides two options for labeling spots: *hierarchic* and *global*.

*Hierarchic labeling* respects the defined array levels and connects the labels of each level to the label of the spot.

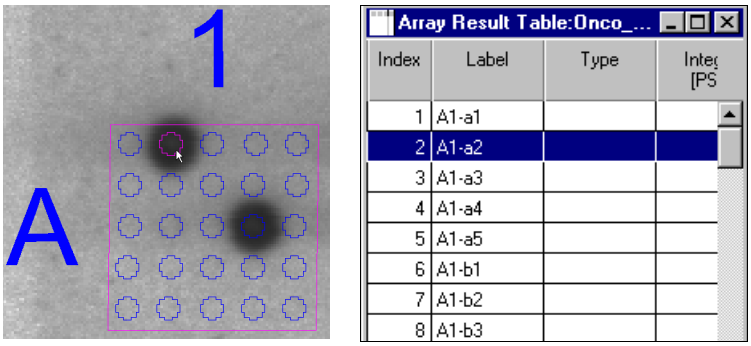



*Figure 3-43 Hierarchic Labeling Example 1*

The hierarchic labels shown in the screenshot example above are composed of two letter-number identifiers representing the two levels of the array. The first identifier (A1) denotes the block in the first row (A) of the first column (1) of the field matrix, the second identifier (a2) denotes the spot (third level) in the second row (2) of the first column (a) of block A1 (field matrix).

The settings for this example are the following:

*Table 3-1 Settings for Hierarchic Labeling Example 1*

| | Columns | Rows | Labeling Type | Label Type | |
|---|---|---|---|---|---|
| | | | | Rows | Columns |
| **Field Matrix** | 16 | 24 | Rows by Columns | uppercase letter | number |
| **Block Matrix** | n/a | n/a | n/a | n/a | n/a |
| **Spot Matrix** | 5 | 5 | Rows by Columns (with prefix “-”) | lowercase letter | number |

In addition, it is also possible to label spots using the numbers defined in repetition patterns. This allows you, for example, to identify corresponding clones from the same microtitre plate. To enable this type of spot labeling, select the **Repeat Pattern** option from the labeling type drop-down menu.

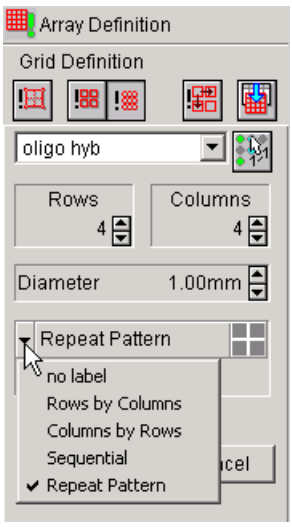



*Figure 3-44 Selecting Repeat Pattern Option*

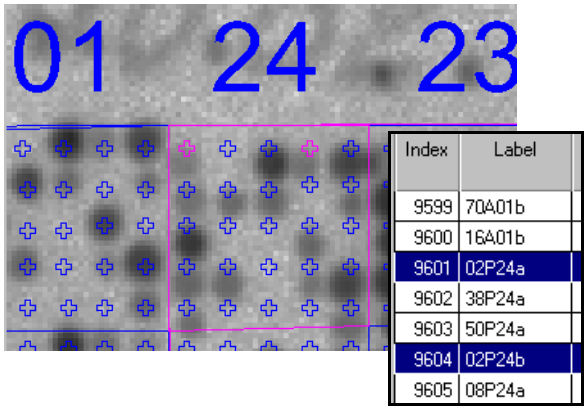



*Figure 3-45 Hierarchic Labeling Example (Repetition Pattern)*

In this example “02” identifies all samples (dots) from microtitre plate number 02, “P24” denotes the wells position on this microtitre plate from which the sample was taken. Since in this example each sample was applied to the filter twice, the suffixes “a” or “b” are used to identify the sample clones.

*Global labeling* ignores the level structure of the array and enumerates the spots according to a given scheme and running direction over the entire array.

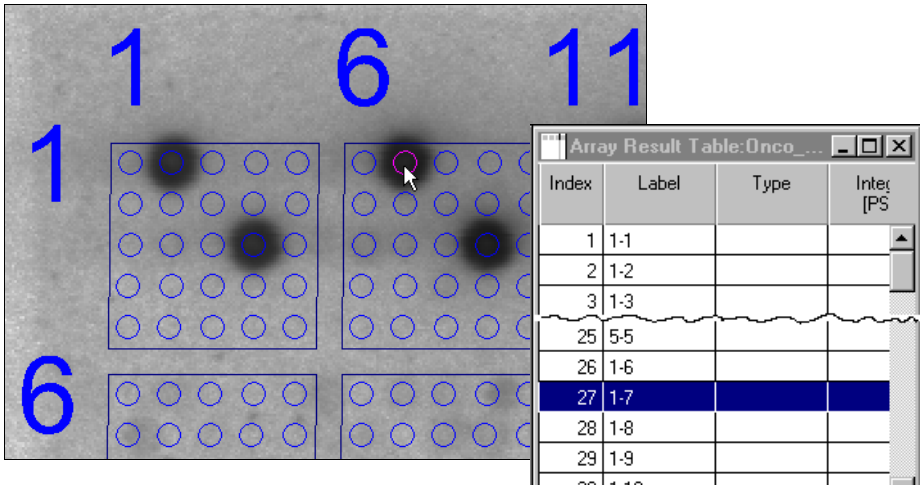



*Figure 3-46 Global Labeling Example*

As shown in the screenshot above, all rows and columns of the array are consecutively numbered. The spot label in this example consists of only one two-part ID number, denoting the global (absolute) position of the spot in the overall array grid (label “1-7” identifies the seventh spot in the first row). As for hierarchic labeling, the substructure of the identifier depends on the labeling scheme for the respective array level chosen from the pull down menus used to define labeling.

**To set the labeling scheme for spots:**

Click the **Define Array Labeling** button in the **Grid Definition** section of the **Array Definition** toolbox.

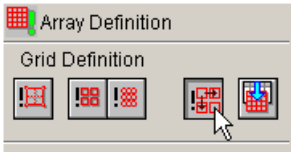



*Figure 3-47 Opening the Array Labeling Dialog*

The **Array Labeling** dialog appears allowing you to specify the labeling type (**Hierarchic Labels** or **Global Labels**).

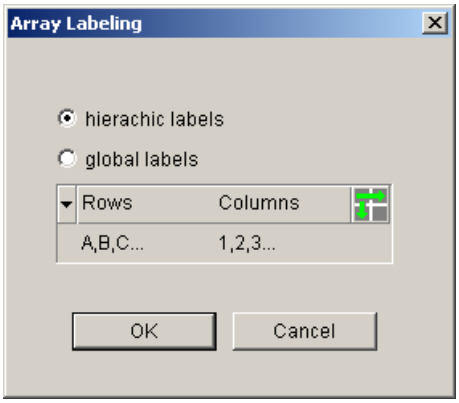



*Figure 3-48 Array Labeling Dialog*

The option for global labels are the same as for array levels (see above).

# Setting Parameters

In some cases, you might want to adjust the default parameter settings for the alignment and/or calculation process. You can do this using the **Settings** panel of the **Array Definition** toolbox. Choose the parameter set you want to change from the **Settings** drop-down list.

## Alignment Settings

If you choose the **Alignment** menu item, the appearance of the lower part of the toolbox changes, revealing control for setting alignment parameters.

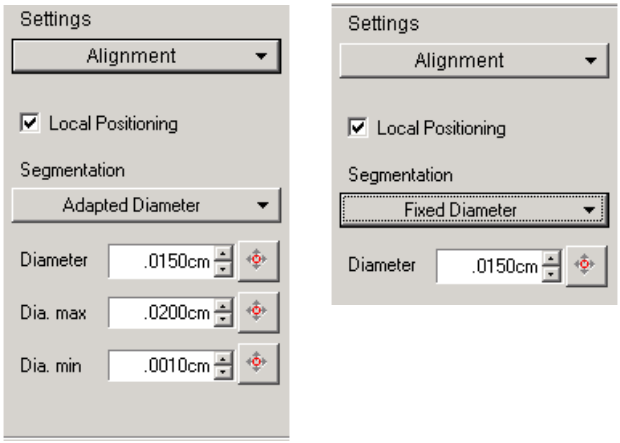



*Figure 3-49 Alignment Controls*

With the **Alignment** controls you can set default parameter values for the automated alignment processes used to match the position of the defined array spots with the real position of the dots in the array. This alignment process is consequently accomplished by the integration of the intensity values of each spot.

The alignment process can be varied by the following parameters:

- **Local Positioning**: Adjusts the position of the array to the image locally.
- **Segmentation**: Choose **Fixed Diameter** to enter a fixed diameter value for all dots or choose **Adapted Diameter** to let AIDA determine the diameter of each dot automatically. If you choose **Adapted Diameter**, you must specify the allowed diameter variation range using the **Dia. max**/**Dia. min** spin boxes.

## Background Settings

If you choose the **Background** menu item, the appearance of the lower part of the toolbox changes, revealing control for setting background parameters.

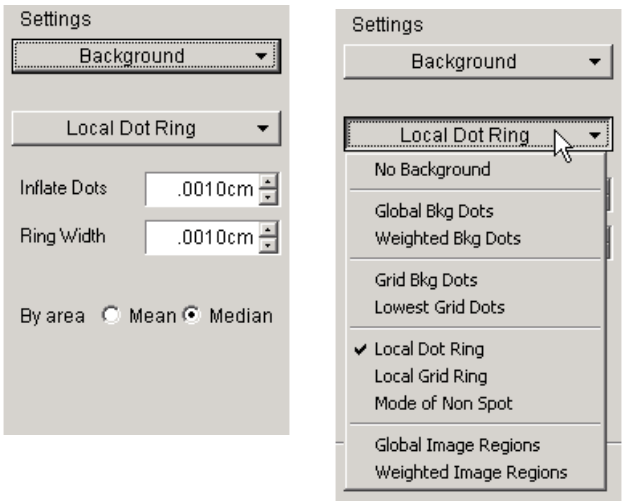



*Figure 3-50 Background Controls*

The intensity values of all spots, which have been aligned and integrated can be corrected for background. Nine different background subtraction methods can be selected from the **Background** menu in the **Settings** section of the **Array Definition** toolbox.

For all these background subtraction methods, you can specify, if the mean or the median value should be used for background calculation. Background spots can be marked by color or label.

## Global Background Dots

The **Mean** or the **Median** value of all selected spots (flagged as “Bkg” or “Empty”) is averaged and the result is used for background subtraction. Consequently the same background value is subtracted from each spot intensity.

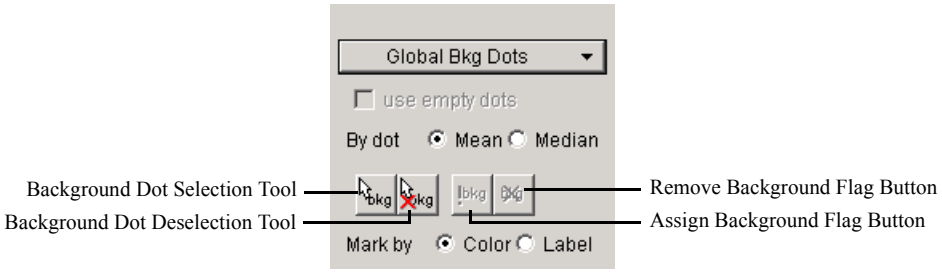



*Figure 3-51 Controls in Global Background Dots Mode*

**To mark a spot for background subtraction:**

1. Choose **Background** from the menu in the **Settings** section of the **Array Definition** toolbox.
2. Choose **Global Bkg Dots** from the menu directly below the **Settings** menu.
3. Choose the **Selection** tool from the **Selection Mode** panel of the **Array Definition** toolbox
4. Click the designated spot
5. Click the **Assign Background Flag** button in the **Settings** section.

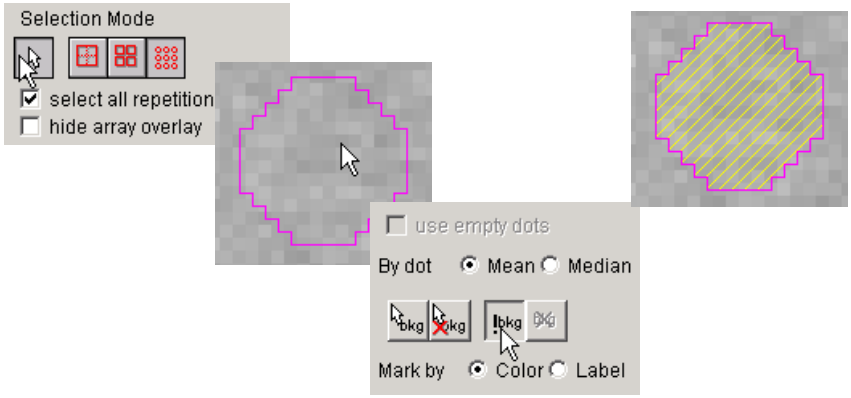



*Figure 3-52 Assigning Background Flags via Assign Background Flag Button*

**Alternative procedure:**

1 Choose **Background** from the menu in the **Settings** section of the **Array Definition** toolbox.

2 Choose **Global Bkg Dots** from the menu directly below the **Settings** menu.

3 Activate the background definition mode by clicking the **Background Dot Selection** tool on the **Settings** panel.

4 Then select a spot in the **Image** window

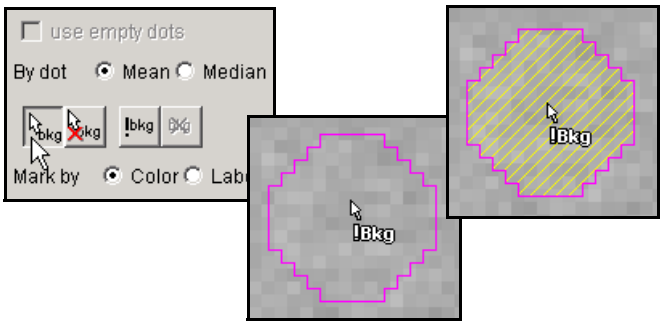



*Figure 3-53 Assigning Background Flag via Background Dot Selection Tool*

In addition, you can specify, whether the background spots are marked by color or by label using the respective controls on the **Background Definition** panel.

**To remove a previously assigned background flag from a spot:**

1 Click the spot(s) you want the background flag to be removed.

2 Click the **Remove Background Flag** button on the **Settings** panel.

**Alternative procedure:**

1 Click the **Background Dot Deselection** tool on the **Settings** panel.

2 Then select the spot in the **Image** window you want the background flag to be removed.

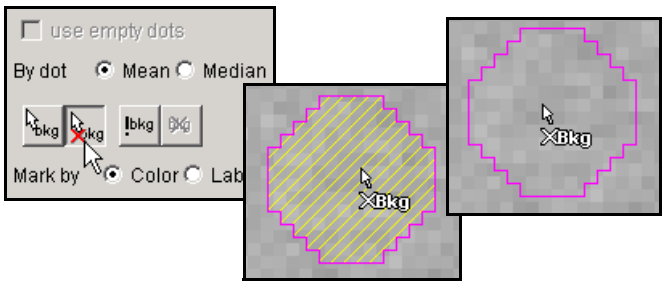



*Figure 3-54 Removing Background Flag via Background Dot Deselection Tool*

If the **use empty dots** option is enabled, the spots, which are defined as empty in the spotting pattern are used for background subtraction.

## Weighted Background Dots

For selecting/deselecting weighted background dots the same selection methods are available as for the selection of global background dots. All selected dots are interpolated for background subtraction. Consequently the background subtraction is fitted to a locally varying background situation. The influence of each background dot on any dot is weighted by the distance between those two dots.

As for the global background dots method, if the **use empty dots** option is enabled, the spots, which are defined as empty in the spotting pattern are used for background subtraction.

## Grid Background Dots

The use of the selected dots for background subtraction is restricted to the sub grid where they are located. The **Mean** or the **Median** values of the selected background dots (flagged as “Bkg” or “Empty”) within a sub grid are averaged and then applied as background to all dots of the sub grid.

## Lowest Grid Dots

If this method is selected, the dots with lowest **Mean** or **Median** values within a sub grid are taken for local background subtraction. They are averaged and then applied to all dots of the sub grid.

Specify the number of minimum intensity dots to be used for background subtraction in the **Take Lowest** spin box.

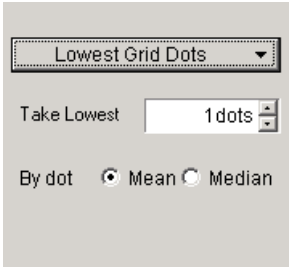



Figure 3-55 Specifying Lowest Dots

## Local Dot Ring

Background can also be subtracted directly around the overlay spot. This results in an individual background determination of each subjected spot.

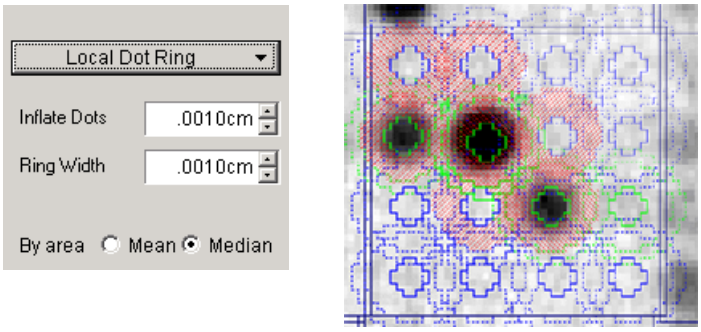



*Figure 3-56 Specifying Local Dot Ring*

A zone around the overlay spot can be defined, where no background subtraction value is measured. This is especially helpful, if spots with high intensities radiate above the measurement spot. To define the zone, enter an appropriate value in the **Inflate Dots** spin box on the **Settings** panel of the **Array Definition** toolbox.

If the Adaptive Diameter Segmentation is enabled, the spot diameter value can differ from spot to spot, but since the **Inflated Dots** value is added to the **Dia. max** value (see the "Alignment Settings" section), all *inflated dots* have the same diameter value.

With **Ring Width**, a distance around the measurement spot and the zone, excluded from background subtraction, can be defined for determination of the background intensity. The **Mean** or the **Median** value of all the pixels within the ring is subtracted from an individual spot.

Note that the inflated dot regions of the neighboring dots, which intersect with the local dot ring, do not enter into the background calculation.

## Local Grid Ring

The **Mean** or **Median** value of all the pixels in a zone around the area of the smallest grid level is taken for background subtraction. As for the local dot ring method, a zone around the overlay spot can be defined, where no background subtraction value is measured. This zone is specified using the **Inflate Dots** spin box.

If the Adaptive Diameter Segmentation is enabled, the spot diameter value can differ from spot to spot, but since the **Inflated Dots** value is added to the **Dia. max** value (see the "Alignment Settings" section), all *inflated dots* have the same diameter value.

All spots of one sub grid are treated by the same background subtraction.

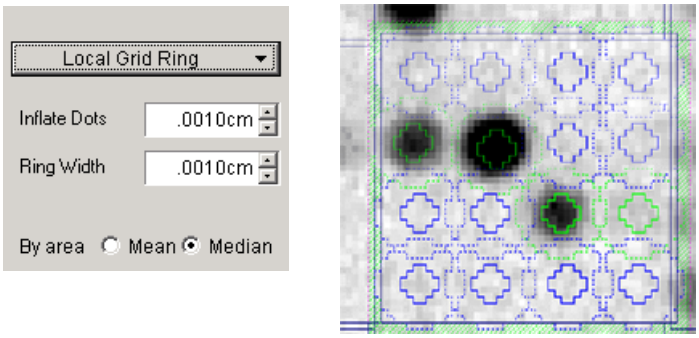



*Figure 3-57 Specifying Local Grid Ring*

## Mode of Non Spot

In **Mode of Non Spot**, the **Mean** or **Median** value of all the pixels in an area within the smallest sub grid around the measurement spots (excluding the spots's pixels) is taken for background subtraction. With **Inflate Dots**, a zone around each spot, where no background is determined can be defined.

If the Adaptive Diameter Segmentation is enabled, the spot diameter value can differ from spot to spot, but since the **Inflated Dots** value is added to the **Dia. max** value (see the "Alignment Settings" section), all *inflated dots* have the same diameter value.

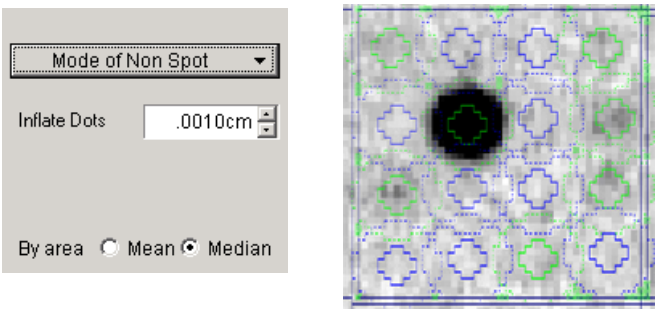



*Figure 3-58 Mode of Non Spot*

## Global Image Regions

Arbitrary rectangles can be defined, which are used globally for background subtraction. This done by averaging the **Mean** or the **Median** values of all the pixels within the arbitrary rectangle and applying the resulting value to all dots. If you define more than one background region, the values of all the regions defined are averaged.

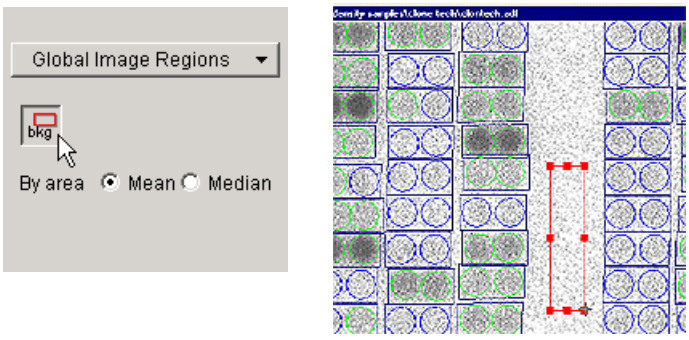



*Figure 3-59 Specifying Global Image Regions*

## Weighted Image Regions

As for **Global Image Regions**, arbitrary rectangles can be defined, which you can use for weighted background subtraction (for differences between global and weighted background subtraction, see the Global and Weighted Background Dots section above).

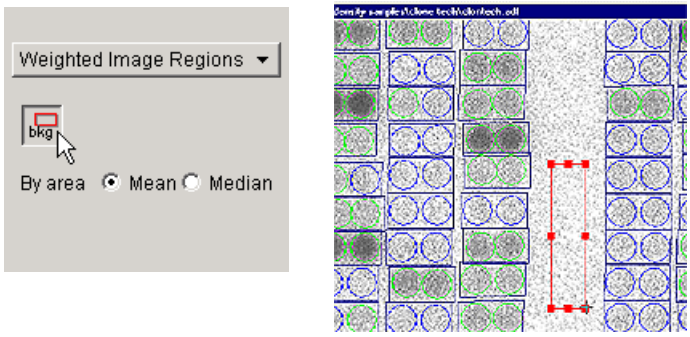



*Figure 3-60 Specifying Weighted Image Regions*

It is strongly recommended that you define as many regions as possible to enhance background subtraction. Also, distribute the regions over the array as evenly as possible. If this is not possible, try to position the regions all around the respective array.

## Rating Settings

If you choose the **Rating** menu item, the appearance of the lower part of the toolbox changes, revealing control for setting rating parameters.

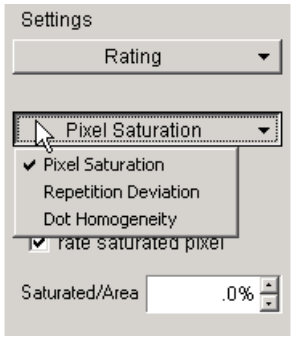



*Figure 3-61 Rating Controls*

The **Rating** controls allow you to set values for the following parameters for the automatic rating process:

- **Pixel Saturation**: Check **rate saturated pixel** to enable the rating of saturated pixels. Enter a threshold percentage of saturated pixels per area. If the number of saturated pixels per area exceeds this threshold, the spot is marked as “bad” and tagged with the label “sat.” (for “saturated”) in the **Quality** column of the result tables..

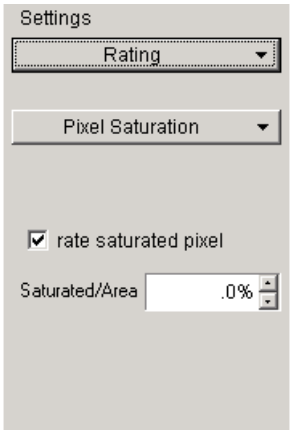



*Figure 3-62 Pixel Saturation Controls*

- **Dot Homogeneity**: AIDA Microarray provides a automatic signal intensity homogeneity check function for spots. The check is performed by measuring the signal intensity distribution within individual spots. The **Diameter** spin box is used to specify the spot area in percentage of the measurement spot to which the check function is applied. Using the **Deviation >** spin box, you can set a percentage of deviation from homogeneous intensity distribution. To set a threshold intensity (in multiples of background), specify the appropriate value in the **Integral >** spin box. To activate the rating of dot deviations, select the **rate dot deviation** checkbox Spots identified by this function are flagged **sd.** ('strong deviation') in the **Quality** row of the **Array Result Table**/**Analysis Table** and the dot deviation value is listed in the **Homogeneity** column.

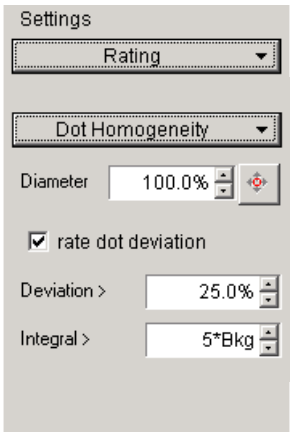



*Figure 3-63 Dot Homogeneity Controls*

- **Repetition Deviation**: This options allow you to flag dot repetitions, which deviate from the average value of the measurement type selected for normalization. Checking the **above probe median** and/or **below probe median** options enables flagging of deviating dot repetitions above and/or below probe median. To set the percentage of deviation from the median, use the **Deviation >** spin box, and to set a

threshold for the median value (given in multiples of the background), specify the appropriate value in the **Median** spin box. This restricts the application of the dot repetition flagging function to spots, which are substantially above background.

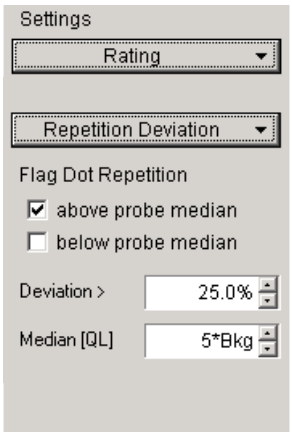



*Figure 3-64 Repetition Deviation Controls*

## Normalization Settings

If you choose the **Normalisation** menu item, the appearance of the lower part of the toolbox changes, revealing control for setting normalisation parameters.

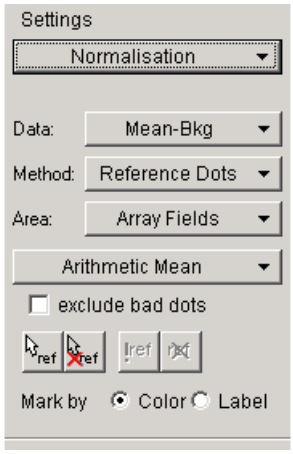



*Figure 3-65 Normalisation Controls*

The **Normalisation** controls allow you to set values for the following parameters:

- **Data**: This menu allows you to select, which type of measurement value should be used for normalisation:

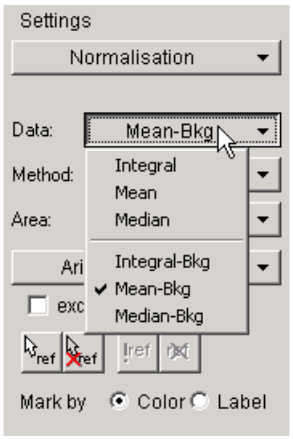



*Figure 3-66 Choosing Measurement Type for Normalization*

- **Method**: For the comparability of the results of two arrays, a normalization of an individual result set is necessary. AIDA Microarray offers two methods of normalization, which can be selected from the **Method** menu of the **Array Definition** toolbox's **Settings** section.

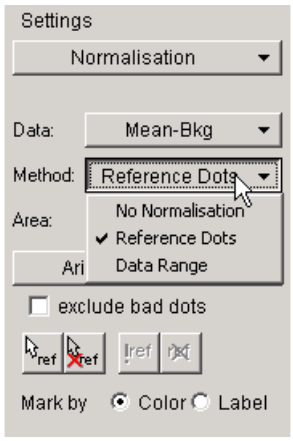



*Figure 3-67 Selecting Normalization Method*

After the normalization process is completed, additional columns (**Normalized**, **Reference**) are inserted in the **Array Result Table**. For details about this columns, see the "Array Result Table" section of this user's manual.

## Reference Dots Normalization Method

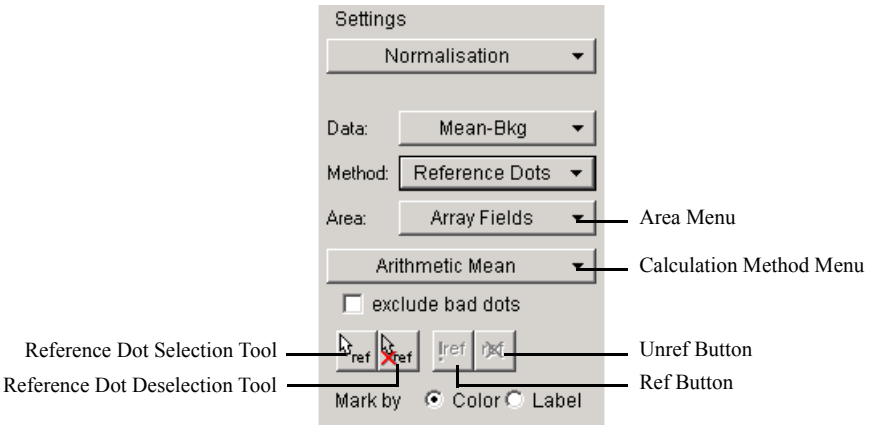



*Figure 3-68 Reference Dots Controls*

There are three normalization domains available, which can be selected using the **Area** menu: **Entire Array**, **Array Field**, and **Array Block**. If you choose **Entire Array**, the reference value results from averaging the values of all reference spots in the entire array. **Array Field** averages the values of all reference spots in an array field, whereas **Array Block** averages the values of all reference spots in an array block.

Each normalization result of a spot is referred to either by the **Arithmetic Mean**, **Geometric Mean**, or the **Variance Stabilisation** value of all spots or to individually selected normalization spots. Normalization is done using the same reference value for all spots. See the "Formal Description of Normalization" section in the Appendix for details.

"Bad" dots can be excluded from normalization by checking the **exclude bad dots** option.

**To mark a reference spot (normalization spot):**

1. Choose the **Selection** tool from the **Selection Mode** panel of the **Array Definition** toolbox
2. Click the designated spot
3. Click the **Ref** button on the **Settings** panel.

**Alternative procedure:**

1. Activate the reference dot definition mode by clicking the **Reference Dot Selection** tool on the **Settings** panel.
2. Select a spot in the **Image** window

**To remove a previously assigned reference flag from a spot:**

1. Click the spot(s) you want the reference flag to be removed.
2. Click the **Unref** button on the **Settings** panel.

**Alternative procedure:**

1. Click the **Reference Dot Deselection** tool on the **Settings** panel.
2. Select the spot in the **Image** window you want the reference flag to be removed.

Reference spots can by marked by color or label. Just specify the marking by selecting one of the **Mark by** radio buttons.

## Data Range Normalization Method

*Data Range* represents a normalization method, where guide dots, empty dots and dots with low/high signal intensity can be excluded from referencing.

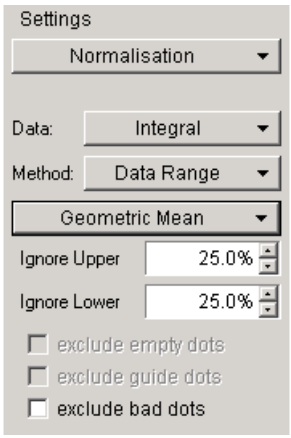



*Figure 3-69 Data Range Normalization Method*

**Data Range** is a global normalization method, normalizing all spots using the same reference value. Each normalization result of a spot is referred to either by the **Arithmetic Mean** or the **Geometric Mean** of the reference spot. See the “Formal Description of Normalization” section in the Appendix for details.

## Ratio Comparison Settings

If you choose the **Ratio Comparison** menu item, the appearance of the lower part of the toolbox changes, revealing control for calculating the ratios of selected data.

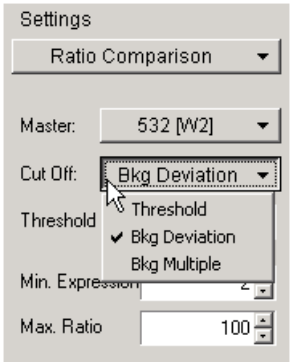



*Figure 3-70 Ratio Comparison Controls*

- **Master**: Specifies the master channel for the comparison. The ratios resulting from the comparison are calculated by dividing the measurement value of the measurement channel by the measurement value of the master channel.
- **Cut Off**: Sets the cut off method (**Threshold**, **Bkg Deviation** or **Bkg Multiple**). The cut off method defines a threshold. If a spot's value is below this threshold, the spot value is regarded as not detected.

  Calculating ratios of data values within background range results in biased expression values, since all measured data values exhibit a certain amount of random error due to the inherent bias of the cloning, array production, array processing, spotting and scanning procedures. Therefore, values within background range oscillate around 0. Forming ratios using those biased background range values just yields results comprised entirely of biases. To filter such biased ratios, you can specify thresholds for the background range using the following controls:

  - **Threshold** specifies a constant background threshold value (indicated by two axis parallel yellow lines in the scatter plot). If the Normalized value of a spot is below this threshold value, the spot value is regarded as not detected.
  - **Bkg Deviation** specifies a threshold for each channel in terms of multiples of the standard deviation of the background values. In the scatter plot, the spots, whose Normalized values are below the threshold of both the measurement and the comparison channel respectively, are displayed in yellow. The threshold is calculated as follows: On performing normalization using the selected normization method, Normalized values as well as normalized background

values (Bkg Normalized) are calculated. The mean and the standard deviation of the normalized background values for the spots are calculated. If the measurement value is background corrected, the threshold is defined by the selected multiple of the standard deviation. If the measurement value is not background corrected, the threshold value is defined by adding the mean of the normalized background values to the selected multiple of the standard deviation.

- **Bkg Multiples** defines a threshold, which requires, that Normalized values must be above a selected multiple of the normalized background (*Normalized* > *n*·*Bkg Normalized*) to be regarded as detected. This method is useful, if the standard deviation of the background signals can be determined only badly or not at all, and the Bkg Deviation method therefore is not applicable. This is usally the case, when you use one of the global methods for background determination.

- **Min. Expression**: Defines an upper and a lower expression threshold.
- **Max. Ratio**: Setting a **Max Ratio** value (the highest ratio value possible) can be very useful, if you want to export the comparison data to another program post-processing these data (e.g. for cluster analysis). Since these programs usually accept numerical data only, and since calculation of ratios of values within background range does not yield reasonable results, Microarray sets the ratio value to **Max Ratio**, if the ratio calculation results in infinity. In the majority of cases, it is reasonable, to set the **Max Ratio** value to 100.

Usually, you will review the statistics of the comparison using the correlation plot displayed in the **Array Scatter Plot** window.

In the correlation plot, the vertical and horizontal yellow lines indicate the background thresholds (vertical line: **Master Cut Off**, horizontal line: **Client Cut Off**), whereas the lines shown in non-yellow colors indicate the respective expression thresholds set by the **Min. Expression** spin box on the **Ratio Comparison** panel of the **Array Definition**

toolbox. Two diagonal yellow-colored lines represent the **Max. Ratio** value (Max. Ratio and 1/Max. Ratio) selectable via the **Ratio Comparison** panel of the **Array Definition** toolbox.

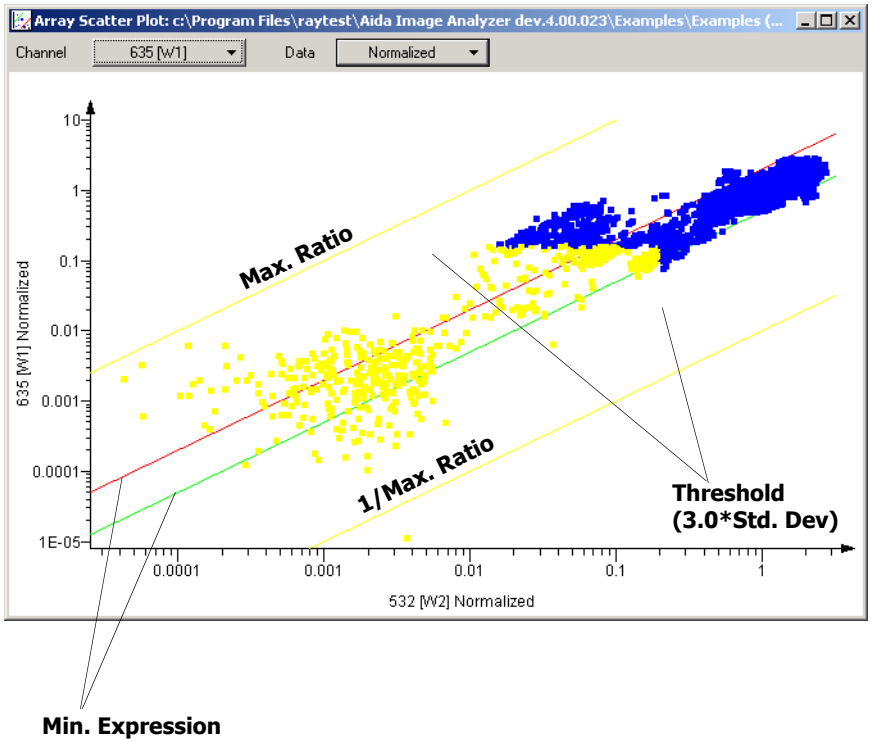



*Figure 3-71 Scatter Plot Representation of Comparison Parameter*

The background threshold divides the scatter plot into four parts:

1. Master value ≤ **Master Cut Off** *and* client value ≤ **Client Cut Off**: Both, master and client value are within the background range, i.e. their ratio cannot be determined. In this case, Microarray sets the ratio value to **1** (expression value: **No**).
2. Master value ≤ **Master Cut Off** *and* client value > **Client Cut Off**: In this case, only the master value is within the background range, whereas the client value is above the background threshold. Their ratio cannot be determined, but since client's measurement value can be determined (in contrast to case 1 above), the ratio value is set to the predefined constant **Max. Ratio** (expression value: **On**).

3. Master value > **Master Cut Off** *and* client value ≤ **Client Cut Off**: This is the opposite case of 2., with the value of the master array above the background threshold and the client array value indeterminable.The ratio value is set to *1/Max. Ratio* (expression value: **Off**).
4. Master value > **Master Cut Off** *and* client value > **Client Cut Off**: Both values are above the background thresholds, so their ratio is determinable (= client value / master value). If ratio value > 1, the expression value is set to *ratio value*, and if ratio value < 1, the expression value is set to *-1/ratio value*.

---

**Note –** For values which are within the range of the expression thresholds (represented by the non-yellow colored lines, and determined by the data range **Min. Expression** spin box settings on the **Ratio Comparison** panel), the real ratio values are calculated in any case. This is due to the fact that small fluctuations in the measurement data causes large differences in interpretation, especially at the intersection point of the **Master Cut Off** and the **Client Cut Off**.
For values which are within the range of the **Max. Ratio** and *1/Max. Ratio* thresholds, the real ratio values are calculated and displayed in the **Ratio** column of the result table, but the **Expression** value is set to **On** or **Off**.

---

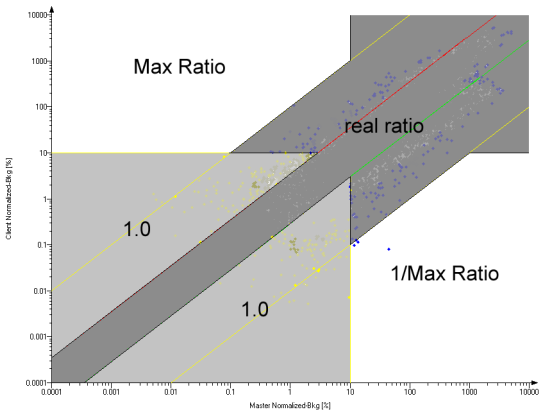



*Figure 3-72 Scatter Plot Partitioned Into Different Ratio Calculation Areas*

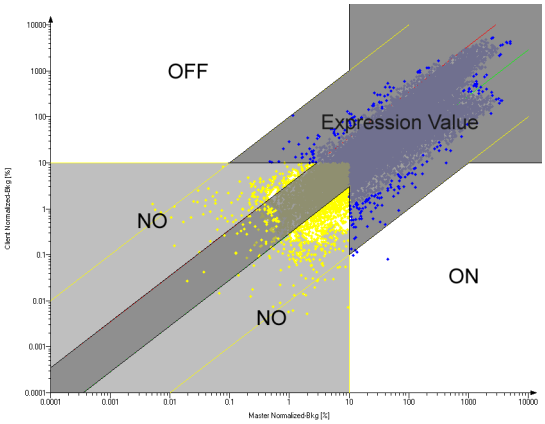



*Figure 3-73 Scatter Plot Partitioned Into Different Expression Value Calculation Areas*

# 3

# Importing Name Tables

The **Name Table Import** option is intended for easy import of names and ID numbers of samples like clones, sequences, oligos and so on. In Microarray you can import up to four columns from a source name table. In order to properly insert the rows of the source columns into matching row slots of the target table (Array Result Table), the position of the particular label column in the source table (import table) must be specified.

Microarray provides four methods for associating table rows:

1. Import by Global Label
2. Import by Hierarchic Label
3. Import by Global Position
4. Import by Hierarchic Positions

The **Import by Global Label** methods associates rows using the **Label** column, **Import by Hierarchic Label** uses the **Field (label)**/**Block (label)**/**Spot (label)** columns, **Import by Global Position** utilizes the **Row Index** and **Column Index** columns, whereas the **Import by Hierarchic Positions** method associates source and target rows via the **Field Row**/**Block Row**/**Spot Row** and **Field Column**/**Block Column**/**Spot Column** columns.

**To import Name Tables:**

**1** Perform one of the following tasks:

- Click the **Name Table Import** button in the **Grid Definition** section of the **Array Definition** toolbox.
- Click the **Import** menu item on the **File** menu and then choose **Array Results** from the cascading menu.

The **Name Table Import** dialog appears.

2 Select the file you want to import from the list of available files and click **Open**.

The file has to be in the Array Name Table or Table ASCII File format.

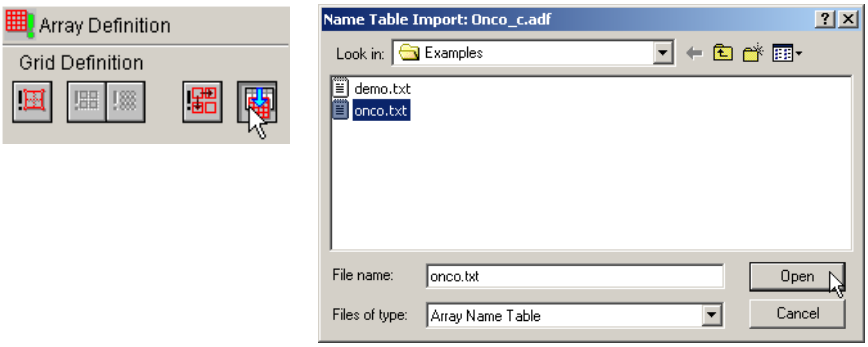



*Figure 3-74 Selecting Name Table File for Import*

The **Import Name Table** dialog appears allowing you to specify the options for the name table import via spin boxes and tabs. In the lower part of the dialog, the import source table is displayed.

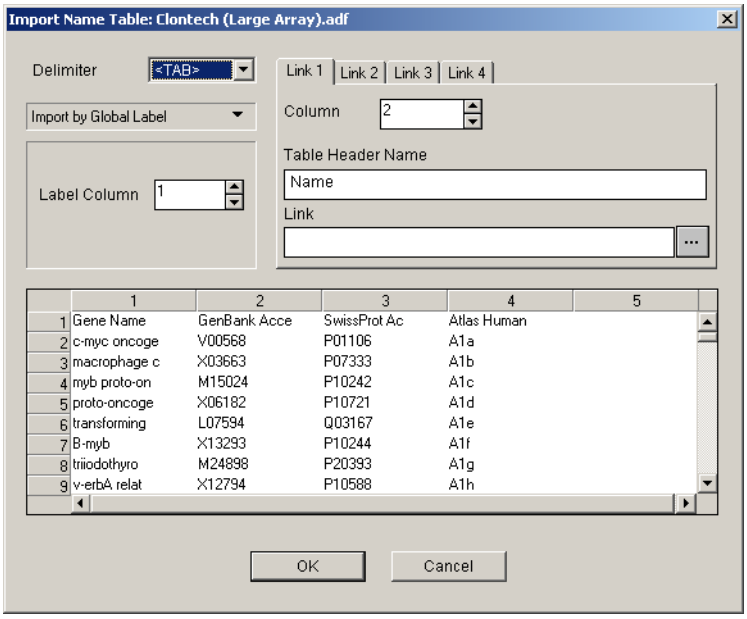



*Figure 3-75 Import Name Table Dialog*

3 Select the type of delimiter (TAB, comma, semicolon or SPACE) from the **Delimiter** drop-down list.

4 Specify the import method using the pull-down menu below the **Delimiter** drop-down list.

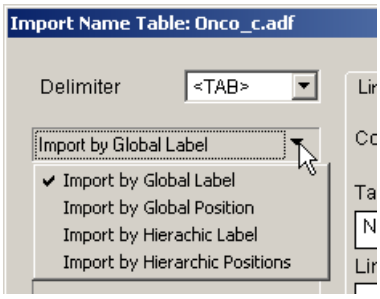



*Figure 3-76 Selecting Import Method*

5 Specify the associations between source table (the name table to be imported) and target table (the actual array result table), depending on the import method you selected:

**If the Import by Global Label method is selected:**

- Specify the position of the label column in the source table using the **Label Column** spin box.

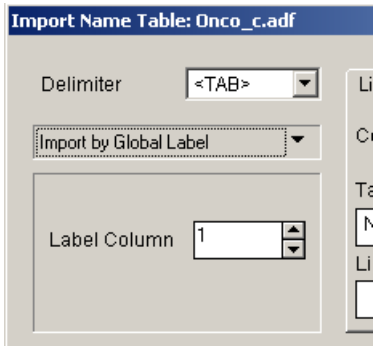



*Figure 3-77 Specifying Label Column Position for the Global Label Method*

**If the Import by Hierarchic Label method is selected:**

- Specify the position of the label column in the source table using the **1.Level Label**/**2.Level Label**/**Spot Label** spin boxes.

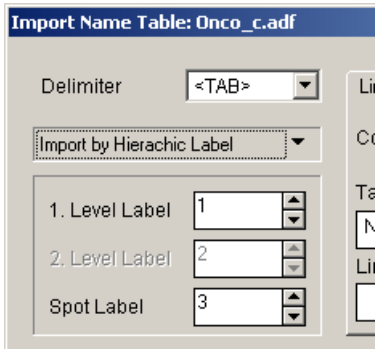



*Figure 3-78 Specifying Label Column Position for the Hierarchic Label Method*

**If the Import by Global Position method is selected:**

- Specify the positions of the column and row indices in the source table using the **Column Index** and **Row Index** spin boxes.

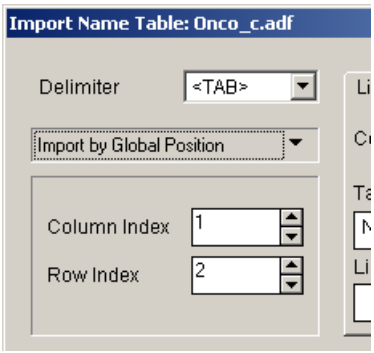



*Figure 3-79 Specifying Index Positions for the Global Position Method*

**If the Import by Hierarchic Positions method is selected:**

- Specify the positions of the column and row indices in the source table using the **Column** and **Row** spin boxes for **1.Level**/**2.Level**/**Spot Level**.

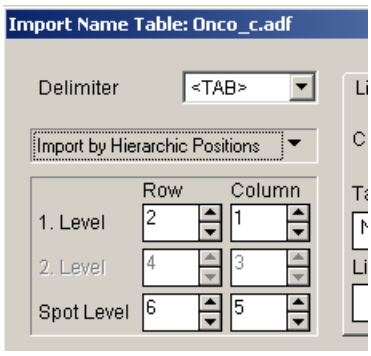



*Figure 3-80 Specifying Index Positions for the Hierarchic Positions Method*

Using the tabbed panel of the **Import Name Table** dialog (**Link 1**, **Link 2**, **Link 3** and **Link 4**) you can specify up to four source columns to be imported into your target table. Furthermore, the tabs allow you to enter a user-defined name for the column header of the resulting target column. In addition, the cells of imported columns (**Name** or **ID** for example) can be hyperlinked to internal or external databases, so you can search the linked databases for the name or ID by just clicking the respective cell in the **Array Result Table**. (Name and hyperlink of imported columns can adjusted via the **Settings of** command selectable from the shortcut menu of the respective column.)

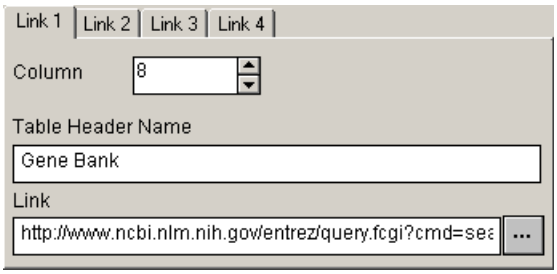



*Figure 3-81 Link Panel*

**6** Enter the number of the source table column you want to import into your target table using the **Column** spin box of the **Link *n*** tab.

Look up the column number in the source table display shown in the lower part of the dialog.

**Note –** Entering the column number 0 in the **Column** spin box of a **Link *n*** tab indicates that no source column has been selected for import on this particular tab.

7 Enter a name for the column to be displayed in the header of the imported column in the **Table Header Name** text box.

8 Enter the query string for database search using database-specific query syntax in the **Link** text box.

Alternatively you can select predefined/previously defined query strings from a list

**a.** Click the button next to the **Link** text box.

The **Table URL Links** dialog opens.

**b.** Choose an entry from the list box and click **OK**.

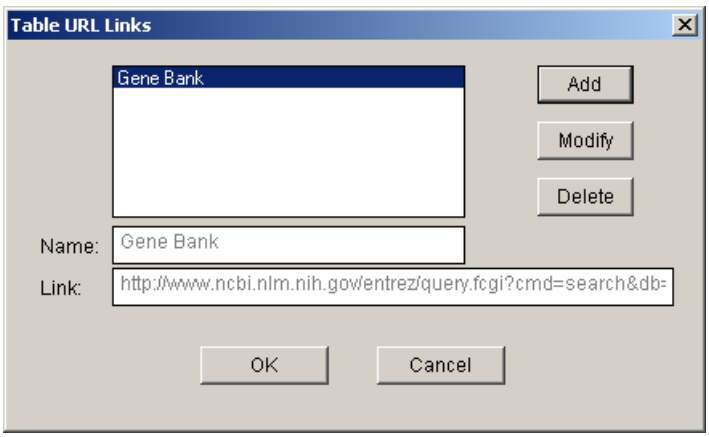



*Figure 3-82 Selecting Query String From the Table URL Links Dialog*

# 3

# Appendix

## Formal Description of Background Subtraction Methods

### Global/Grid Background Dots

Let $B = \{b_i \mid i = (1, \ldots, N)\}$ denote the set of all background spots in the array (or in a block).

A background spot is defined as a spot which either has the background attribute set or is marked as empty (**use empty dots** option checked).

Let $Signal$ denote the Mean or Median value $SD$ the standard deviation of the pixel intensity values for a spot.

The background for all spot in the array (or block) is calculated as follows:

$$Bkg = \frac{\sum (Signal\,(b_i) \cdot Area\,(b_i))}{\sum Area\,(b_i)} \tag{1}$$

$$BkgSD = \frac{\sum (SD\,(b_i) \cdot Area\,(b_i))}{\sum Area\,(b_i)} \tag{2}$$

### Weighted Background Dots

Let $Signal$ denote the Mean or Median value and $SD$ the standard deviation of the pixel intensity values for a spot.

Let $B = \{b_i \mid i = (1, \ldots, N)\}$ denote the set of all background spots in the array.

A background spot is defined as a spot which either has the background attribute set or is marked as empty (**use empty dots** option checked).

Let $d_i\,(spot) = |Position\,(spot) - Position\,(b_i)|$ denote the Euclidean distance between a spot and the $i^{th}$ background spot.

The weighted background for a spot is calculated as follows:

$$Bkg = \frac{1}{D} \sum \frac{(Signal\,(b_i) \cdot Area\,(b_i))}{d_i\,(spot)} \tag{3}$$

$$BkgSD = \frac{1}{D} \sum \frac{(SD\,(b_i) \cdot Area\,(b_i))}{d_i\,(spot)} \tag{4}$$

where

$$D = \sum \frac{Area\,(b_i)}{d_i\,(spot)} \tag{5}$$

**Note** – If all spots of an array have the same shape and diameter, the area normalization has no influence on the background calculation.

### Weighted Image Regions

Let $Signal$ denote the Mean or Median value and $SD$ the standard deviation of the pixel intensity values for a given region on the image.

Let $B = \{rg_i \mid i = (1, \ldots, N)\}$ denote the set of all background regions in the array.

Let $d_i\,(spot) = |Position\,(spot) - Position\,(rg_i)\,|$ denote the Euclidean distance between a spot and the $i^{th}$ background region.

The weighted background for a spot is calculated as follows:

$$Bkg = \frac{1}{D} \sum \frac{(Signal\,(rg_i) \cdot Area\,(rg_i))}{d_i\,(spot)} \tag{6}$$

$$BkgSD = \frac{1}{D} \sum \frac{(SD\,(rg_i) \cdot Area\,(rg_i))}{d_i\,(spot)} \tag{7}$$

where

$$D = \sum \frac{Area\,(rg_i)}{d_i\,(spot)} \tag{8}$$

### Global Image Regions

Let $Signal$ denote the Mean or Median value and $SD$ the standard deviation of the pixel intensity values for a given region on the image.

Let $B = \{rg_i \mid i = (1, \ldots, N)\}$ denote the set of all background regions in the array.

The background for a spot is calculated as follows:

$$Bkg = \frac{\sum (Signal\,(rg_i) \cdot Area\,(rg_i))}{\sum Area\,(rg_i)} \tag{9}$$

$$BkgSD = \frac{\sum (SD\,(rg_i) \cdot Area\,(rg_i))}{\sum Area\,(rg_i)} \tag{10}$$

$$D = \sum \frac{Area\,(rg_i)}{d_i\,(spot)} \tag{11}$$

### Lowest Grid Dots

Let $B = \{b_i \mid i = (1, \ldots, N)\}$ denote the set of spots in a block with the $N$ lowest signal values.

Let $Signal$ denote the Mean or Median value and $SD$ the standard deviation of the pixel intensity values for a spot.

The lowest grid dot background for the spots in the block is calculated as follows:

$$Bkg = \frac{\sum (Signal\,(b_i) \cdot Area\,(b_i))}{\sum Area\,(b_i)} \tag{12}$$

$$BkgSD = \frac{\sum (SD\,(b_i) \cdot Area\,(b_i))}{\sum Area\,(b_i)} \tag{13}$$

### Local Dot Ring

For each spot a circular area (*ring*) around the spot is taken into account for the calculation of background of spot. Neighbouring spot areas are clipped from the ring. The Bkg value is either the Mean or Median of pixel intensities in this area, and the BkgSD their standard deviation.

### Local Grid Ring

Around each block a circular area (*ring*) is taken into account for the calculation of the background for all spots in the block. The Bkg value is either the Mean or the Median of pixel intensities in this area, and the BkgSD their standard deviation.

### Mode of Non Spot

For each block in the array the "non spot" area is the block area without the spot areas. The spot areas might be inflated to avoid overshining problems. The Bkg value for all spots in the block is either the Mean or the Median of pixel intensities in the "non spot" area and the BkgSD their standard deviation.

## Formal Description of Normalization

### Normalization by Reference Dots

Let $Signal$ denote the measurement value of a spot which is used for the normalization.

Let $R = \{r_i \mid i = 1, \ldots, N\}$ denote the set of all reference dots within the entire array, a field, or a block.

For any spot within the entire array, field or block respectively the Normalized value is calculated by

**Arithmetic Mean:**

$$C = \frac{1}{N} \sum Signal\,(r_i) \tag{14}$$

$$Reference\,(spot) = C \tag{15}$$

$$Normalized\,(spot) = \frac{Signal\,(spot)}{C} \tag{16}$$

**Geometric Mean:**

$$C = \exp\left(\frac{1}{N} \sum \left(\log\left(Signal\,(r_i)\right)\right)\right) \tag{17}$$

$$Reference\,(spot) = C \tag{18}$$

$$Normalized\,(spot) = \frac{Signal\,(spot)}{C} \tag{19}$$

**Variance Stabilization:** The Variance Stabilisation Normalization for microarray data was proposed by Wolfgang Huber et al. (2002) in the paper "Variance stabilization applied to microarra data calibration and the quantification of differential expression"[1].

This normalization method requires two or more channels which are normalized simultaneously. The parameter estimation in AIDA follows the same robust maximum likelihood estimation, which is mentioned in the above papers. In fact, in AIDA the "optim" function with method "L-BFGS-B" of the statistical R package is integrated. In the current version of AIDA only the parameter estimation step for known not regulated genes of the Variance Stabilisation Normalisation is implemented. The papers include a second iteration step for selecting not regulated genes, which is not implemented yet. Instead of this, the set of not regulated genes is given by the set of reference dots.

For all channels $i = 1, \ldots, d$ in the array, let

$$h_i\,(spot) = \operatorname{arcsinh}\left(a_i + b_i \cdot Signal\,(spot)\right) \tag{20}$$

denote the variance stabilization transformation proposed in Huber et al. (2002), which is calculated from the set of reference dots as not regulated genes.

[1] Huber, W./von Heydebreck, A./Sültmann, H./ Poustka, A./Vingron, M. (2002): "Variance stabilization applied to microarra data calibration and the quantification of differential expression". *Bioinformatics* 18 Suppl. 1. 96–104.

Than

$$LogNormalized\,(spot) = h\,(spot) \tag{21}$$

$$Normalized\,(spot) = 2^{(h(spot))} \tag{22}$$

$$Reference\,(spot) = not\ defined \tag{23}$$

**Normalization by Data Range**

Let *Signal* denote the measurement value of a spot which is used for the normalization.

Let $D = \{d_i \mid i = 1, \ldots, N\}$ denote the set of applied measurement dots within the entire array. After sorting $D$ by signal values, percentages of the amount $N$ are rejected from the small and the large values side respectively. This leads to the set $D' = \{d'_i \mid i = 1, \ldots, N'\}$ of dots which are used for calculating a global normalization factor for the array channel.

**Arithmetic Mean:**

$$C = \frac{1}{D} \sum Signal\,(d'_i) \tag{24}$$

$$Reference\,(spot) = C \tag{25}$$

$$Normalized\,(spot) = \frac{Signal\,(spot)}{C} \tag{26}$$

**Geometric Mean:**

$$C = \exp\left(\frac{1}{N} \sum \left(\log\left(Signal\,(d'_i)\right)\right)\right) \tag{27}$$

$$Reference\,(spot) = C \tag{28}$$

$$Normalized\,(spot) = \frac{Signal\,(spot)}{C} \tag{29}$$